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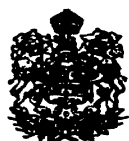
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TABLE I

EFFECT ON THE VIRULENCE¹ OF *Rhizoctonia Solani* ON POTATO SPROUTS PRODUCED BY DIFFERENT FRACTIONS OF SOIL INOCULUM

Expt.	Year	Temp. °C.	Isolate	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	Soil inoc.		Soil control
								A ²	B ³	
Soil inoculum in a cultivated soil										
1	1933	18	48		17	10	0			Tr.
2	1933	18	76		60	51	24			0
2	1933	18	48		10	1	0			0
3	1933	17	76		55	35	23			Tr.
3	1933	17	48		9	9	7			0
4	1934	18	76		75	81	44			0
4	1934	18	106		50	21	15			0
4	1934	18	48		10	1	0			0
5	1935	16	76		50	45	37			0
5	1935	16	106		43	37	8			0
6	1935	17	76		15	8	5			0
6	1935	17	106		30	5	8			0
6	1935	17	48		15	2	0			0
7	1935	16	76		67	63	38			Tr.
7	1935	16	106		20	14	0			0
7	1935	16	48		7	1	3			0
Soil inoculum in a virgin soil										
8	1934	24	106		15	0	1			0
9	1935	17	106	61					0	0
9	1935	17	56	52					Tr.	0
9	1935	17	76	58					4	0
10	1937	16	76	77						0
11	1938	16	76	70					1	0
12	1939	16	76	50					2	0
Soil inoculum in a steam sterilized soil										
13	1937	16	76		0	5	Tr.			0
14	1940	16	76							0
Steam sterilized soil in a naturally infested cultivated soil										
15	1933	17			67	45	40			72
16	1935	16			20	14	5			27
17	1936	16			5	1	0			11

¹ Disease rating per cent, based on 20 plants.² Steam sterilized soil inoculated seven days after sets were planted.³ Steam sterilized soil permeated by vigorous growth of pathogen when sprouts emerged from sets.

The results obtained in the various tests of the two series just outlined, and listed in Table I, indicate that, as a rule, there was definitely less disease and the host more frequently escaped attack when it emerged into soil already permeated by vigorous growth of the pathogen than when host and parasite



FIG. 1. These photographs illustrate the decrease of virulence and the increase of sclerotia formation by *Rhizoctonia Solani* in pure inoculum grown in steam sterilized soil. Isolate No. 106 (left) exhibits moderate virulence in a 1 : 15 soil-inoculum-natural-soil mixture, and isolate No. 76 (right) is very virulent.

met midway in the soil as a result of the delayed inoculation mentioned. However, severity of disease was much reduced and the proportion of plants that escaped attack in either kind of soil inoculum was far greater than in the corresponding series containing 1 part of sterilized soil inoculum and 15 parts of virgin soil.

The addition of steam sterilized soil to a natural soil infested with virulent races of *R. Solani* seems to have somewhat the same tendency to depress virulence as soil-grown inoculum has when added to virgin or cultivated soil (Experiments 15, 16, and 17 (Table I)). Also, the virulence of the soil inoculum of isolate No. 76, when mixed with steam sterilized soil, was very definitely reduced in Experiments 13 and 14.

Effect of Massing of Mycelia and Sclerotia Formation

In inoculated steam sterilized soil, or when large fractions of steam sterilized soil were mixed with virgin soil and inoculated, the formation of sclerotia and the massing of mycelia on the stems were very common and often heavy at normal soil moisture. However, in the 1 : 15 inoculum and natural soil mixture, these formations were much less frequent, and usually not so pro-

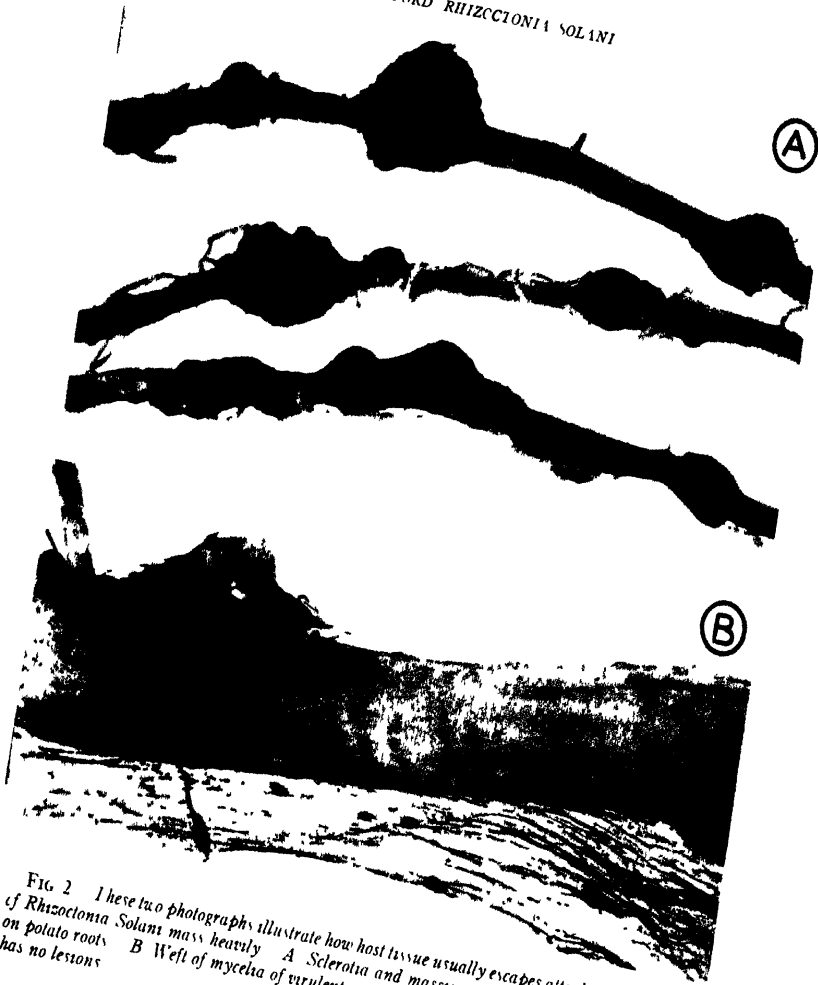


FIG. 2 These two photographs illustrate how host tissue usually escapes attack when mycelia of *Rhizoctonia Solani* mass heavily. A Sclerotia and massing of mycelia of isolate No 106 on potato roots. B West of mycelia of virulent isolate No 76 pulled aside. Note young stem has no lesions.

Moreover, it was rather exceptional for the mycelia to mass or sclerotia to form on the roots in inoculated natural soil. On the other hand, this sometimes happened in inoculated steam sterilized soil (Fig. 2, A). With other factors constant and favourable for vigorous hyphal growth, the formation of sclerotia was especially favoured when the water content of the soil was slightly above optimum, and as a general rule distinctly inhibited when the soil was rather dry, a fact that agrees with evidence presented earlier. On the other hand, it was found that (2) sclerotia sometimes formed in a soil with a water content of 21% of its water-holding capacity. Although sclerotia formed most readily on the new shoots, the periderm of the parent set provided a substrate almost as favourable.

The evidence indicates that sclerotia formation is very closely associated with sudden growth of new hyphae. Hyphae even a few days old seem to have lost the ability to initiate sclerotia. Very important among the non-living factors are an adequate supply of nitrate nitrogen (3), a high relative humidity in the soil interstices, and a soil temperature range of 16° to 20° C., as demonstrated by this and previous studies. Of these, an optimum relative humidity appears to be the most important single factor. On the other hand, the sclerotia often did not form in the inoculated, steam sterilized loam used, when its water content was optimum for best growth of plants and mycelium.

Certain isolates of *R. Solani* characteristically produce sclerotia much more readily than others under identical conditions. For example, isolates No. 106, obtained from a single basidiospore of the *Corticium* stage, and No. 48, from a sclerotium on a potato tuber, were both far less virulent than isolate No. 76, which was isolated from a disease lesion on a potato stem. Isolates No. 106 and 48 both produced sclerotia and massed hyphae on the shoots much more abundantly and frequently than isolate No. 76.

The marked difference between isolates No. 106 and 76 in ability to form sclerotia, and in pathogenicity under apparently identical conditions, in soil-grown inoculum protected from outside contamination, is shown in Fig. 1. These are typical of the 20 plants in each lot.

Effect of Age of Inoculum on its Virulence

The effect of age and condition of culture has been discussed by several workers without agreement as to whether a young culture is more virulent than an old one. In general, however, these discussions seem to refer to the age of the culture in test tubes rather than to the age of inoculum as prepared in bulk for experiments in the soil. In the absence of satisfactory evidence on this important question, similar experiments were completed during 1937, 1938, and 1939. Each year inoculum of No. 76, a very virulent isolate of *R. Solani*, was increased in sterilized black loam in Erlenmeyer flasks, as described under "Methods". Thus, there were obtained different lots of inoculum, the age of which varied from 6 to 180 days. As soon as the myce-

TABLE II
EFFECT OF AGE OF INOCULUM ON VIRULENCE OF *Rhizoctonia Solani* ON POTATO

Year	Average ¹ disease rating, %							Temp., °C.	Soil moisture ²
	Age of inoculum in days								
	180	150	120	90	60	30	6		
1937	—	60	63	57	57	54	57	17	28
1938	50	60	42	61	60	56	52	17	28.6
1939	—	40	42	41	40	46	40	16	29

¹ Based on 20 plants, each in separate flask.

² Per cent moisture holding capacity.

lium had ramified through the soil in the flasks, which usually required a period of about ten days, that particular lot of flasks was stored at approximately 3° C. until used, and the moisture content maintained. In preparing the experiment, 1 part of this soil inoculum was mixed with 15 parts of virgin black loam. The test was made at 16° C. on the Early Ohio variety grown in flasks as described under "Methods". The disease ratings, arising from the inocula of different ages, are listed in Table II. The evidence is very convincing that the soil-grown inoculum six days old was as virulent as that at least 150 days old.

Discussion

The results of the many experiments listed in Table I leave no doubt that *R. Solani* was much more virulent in the inoculated natural black loam used, with its natural complement of microflora and fertility, than in the same soil steam sterilized and inoculated. The data in Experiments 8, 9, and 10 (Table II) prove conclusively that in general, isolates that are normally very virulent in a natural soil with which has been mixed a small portion of soil inoculum are almost certain to produce a very slight amount of disease in soil-grown inoculum not mixed with natural soil.

As the decrease of virulence closely followed the increase of soil inoculum, one suspects that, for most effective infection, there developed too much mycelium of the pathogen introduced, or too great a growth of it afterwards, as a result of increased fertility made available by sterilizing the black loam, or by staling effects of the more abundant mycelial growth of the pathogen. In this connection, the observational evidence from thousands of young potato plants throughout these studies seems to indicate that the pathogen is most virulent when its hyphae are very young, very thin, and still hyaline, and difficult to find on the susceptible host tissue. Indeed, when the mycelium can be easily seen, and especially when it masses more or less heavily, susceptible host tissue is usually afforded complete protection from infection (Fig. 2, B) despite the fact that the isolate is potentially very virulent. This observation has been so consistent in these experiments during seven years, that one must conclude that factors which cause massing of the mycelium also depress its virulence.

Evidence concerning formation of sclerotia on the underground stems of potato plants indicate that these bodies usually accompany sudden and abundant mycelial growth and that the humidity of the soil air is of prime importance. Also, since sclerotia formed quite readily during this and previous (1, 2) experiments at soil temperatures from 15° to 22° C., when the water content of the soil was about 21 or 28 to 30% of its moisture-holding capacity in both the steam sterilized and the natural soil, it seems clear that conditions favourable for sclerotia are not narrowly restricted by temperature or by water content of the soil within the range mentioned. The relative effect of antagonism on sclerotia formation in the two soils mentioned was not determined.

A possible explanation of the equal virulence of the old and new inocula under the conditions of the test is that infection would depend on new growth from bits of mycelium scattered throughout the soil, and that any initial differences in vigour of growth and virulence would have disappeared by the time the sets had sprouted. Probably one should not expect differences to appear in a test of this kind.

In conclusion, these studies throw an interesting light on the general pathogenicity and virulence of *R. Solani* under field conditions. First, there are the important differences in pathogenicity of the various isolates as determined in this and a previous study (1). These differences undoubtedly range from practically zero to very pathogenic. Isolates also differ in ability to form sclerotia. Being a natural and vigorous soil saprophyte, the various isolates of the pathogen increase or decrease in abundance and relative prevalence, depending on local conditions. It also seems likely that the effect of a given crop on the increase or persistence of the various races in the soil is imperfectly understood. In addition to this general situation, is the fact that fertility, water content, or temperature of the soil may markedly reduce or increase the ability of the various races to successfully attack the host. These aspects of the problem are now being examined further in connection with a study of the effects of various food sources on the virulence and persistence of the pathogen.

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THE RELATION BETWEEN FROST RESISTANCE AND THE PHYSICAL STATE OF PROTOPLASM

II. THE PROTOPLASMIC SURFACE¹

By D. SIMINOVITCH² AND J. LEVITT³

Abstract

Deplasmolysis injury, ductility of cytoplasmic strands, and the shape assumed by injected oil drops on deplasmolysis were investigated. The surface membrane of the protoplast of non-hardy cells stiffened when dehydrated osmotically. As a result, it ruptured readily when subjected to tension. The stiffening either failed to occur in hardy cells, or it arose only as a result of a much greater dehydration (depending on the degree of hardness). The refractive index of the protoplasmic surface increased more on dehydration in the case of non-hardy than of hardy cells. Plasmolysis, if maintained for some time, induced a clumping of plastids and granules (systrophy) in non-hardy but not in hardy cells. All these facts indicate a greater hydrophilicity in hardy than in non-hardy cells—both of the surface membrane of the protoplast and, as shown in Part I, of the protoplast as a whole, although it is probably less marked in the latter.

In Part I of this series (7) it was shown that the frost-hardening process produces certain changes in the physical state of protoplast. The methods used, however, revealed the nature either of the protoplast as a whole or simply of the mesoplasm, and yielded little or no information concerning the ectoplasm. This paper is concerned primarily with the properties of the protoplast surface, as revealed by investigations of deplasmolysis injury, ductility of cytoplasmic strands, and the shape of injected oil drops.

Deplasmolysis Injury

Though it has been established by Scarth and Levitt (8) that hardy cells are more resistant to deplasmolysis injury than are non-hardy cells, more information is required in order to establish the exact conditions under which the differences occur and the exact location of the injury. As in previous investigations, plasmolysis, vital staining (with neutral red), and the general appearance of the cytoplasm were used to determine whether the cells were alive.

Effect of Deplasmolysis Rate

Dehardened cortical cells of *Catalpa* and *Cornus* twigs were progressively plasmolysed in stronger and stronger balanced solutions of sodium chloride and calcium chloride (9 : 1) until an osmotic concentration of 200 atm. was reached. They were then partially deplasmolysed in a solution having a concentration of 50 atm. and were finally transferred to water. Though the protoplasts expanded slowly in the water, they all burst before reaching

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the cell wall. This occurred even if deplasmolysis was slowed up still more, by adding water to the plasmolyte drop by drop. If, however, deplasmolysis was not allowed to proceed too far, the protoplasts did not burst.

Hardy cells of *Catalpa* and *Cornus* always deplasmolysed without injury, even when transferred directly to water from the strongest solution.

Similar results were obtained with cane and invert sugar.

Effect of Dehydration and Rehydration in the Absence of Plasmolysis

Cortical sections of *Catalpa* and *Cornus* were transferred directly to very strong sugar solutions (saturated sucrose or supersaturated invert sugar with osmotic pressures of about 200 to 300 atm.). The cells contracted as a whole, the wall collapsing and remaining in contact with the protoplast. This was presumably due to the slow penetration of the sugar molecules into the cell wall. Hardy cells in this state suffered no injury on transfer to water. The protoplasm swelled more or less uniformly on all sides as the whole cell expanded. Non-hardy cells, however, were all killed, except a few in which protoplasts or vacuoles with coagulated protoplasm underwent pseudo-plasmolysis. Even those subsequently succumbed.

These results are summed up in Table I.

TABLE I

SURVIVAL OF DEHARDENED AND HARDY CORTICAL CELLS OF *Catalpa* AND *Cornus* ON TRANSFER FROM HYPERTONIC TO WEAKER SOLUTIONS

Solutes	Osmotic conc. of solutions, atm.	State of cells in strongest solution	Cells surviving	
			Dehardened	Hardy
Sodium chloride + calcium chloride (9 : 1)	50→0	Plasmolysed	Most	All
	50→200→30→0	Plasmolysed	None	All
Invert sugar and sucrose	50→300*→27	Plasmolysed	All	All
	300*→27	Collapsed	Few	All
	27→0	Plasmolysed	All	All
	50→300*→27→0	Plasmolysed	None	All
	300*→27→0	Collapsed	None	All

*Approximate (molar concentration about 6 M).

Effect of Increase in Surface Area during Deplasmolysis

Sections of dehardened *Catalpa* cortex which were cut directly into M sodium chloride and calcium chloride (9 : 1) showed convex plasmolysis in most cells. If, after about half an hour, they were transferred to water, almost all were killed. On the other hand, sections which were first put in hypotonic calcium chloride or dextrose, showed concave plasmolysis on transfer to the above plasmolyte. If they were transferred to water even after more than half an hour in the plasmolyte, most of the cells deplasmolysed quite normally and remained uninjured. Though the dehydration was

exactly the same in both cases, the protoplast surface, being more reduced in the first test, had also to stretch more, and this produced the injury.

Effect of Length of Time in the Plasmolyte

Previous investigations by Scarth and Levitt (8) have shown the importance of the time factor—the longer the sections were left in the plasmolyte, the greater was the deplasmolysis injury. Further tests indicated that hardness can be correlated with the time necessary to leave the sections in a solution before obtaining deplasmolysis injury. *Hydrangea*, *Picea*, and *Catalpa* cells tested during fall were all killed on deplasmolysis after a 10 minute plasmolysis in a solution of 4 *M* sodium chloride and calcium chloride (9 : 1). In early winter, these three species became more hardy and required exposures of 30, 90, and 120 minutes, respectively, to the same plasmolyte before suffering deplasmolysis injury.

On the other hand, as shown in Part I of this series (7), the difference in resistance to deplasmolysis injury in the case of cabbage was slight if both the hardened and unhardened cells were plasmolysed for only a short time. Since the protoplasts rounded up gradually, the extent to which reduction of surface had proceeded might be a factor in the time effect. Experiments with unhardened cabbage throw some light on this question (Table II).

TABLE II
EFFECT OF TIME IN PLASMOLYTE ON DEPLASMOLYSIS INJURY TO UNHARDENED CABBAGE
(Average of three plants)

Dextrose conc., <i>M</i>	Time in plasmolyte	Plasmolysis shape	Cells killed on deplasmolysis, %
1.0	15–20 min.	Mostly concave	25
	2 hr.	Mostly convex	50
	5½ hr.	All convex	90
0.75	15–20 min.	Mostly convex	25
	2 hr.	All convex	40
	5½ hr.	All convex	90

The injury obviously increased with the time in the plasmolyte, even after the protoplasts had completely rounded up. Degree of surface stretch was therefore not a cause of this increased injury. On the other hand, it might be due to a stiffening of the protoplast surface in plasmolysed cells. If deplasmolysis injury is really a surface injury, such a stiffening would explain the time factor. In order to clear up this point, the deplasmolysis process was observed directly.

As already mentioned, a large proportion of cells of unhardened plants, plasmolysed (concavely) in a solution of sodium chloride and calcium chloride with an osmotic concentration of 50 atm. and then transferred to water, were uninjured. Some 25%, however, were killed. Observation of these during deplasmolysis revealed an increase in thickness of the cytoplasm layer followed

by rupture of the outer membrane before deplasmolysis was completed. This allowed escape and dispersion of the protoplasm. The vacuole continued to expand slightly, then it, too, burst. The bursting of the outer membrane occurred either at the side of the protoplast (just where it touches the wall) or at the tip where the cytoplasm layer is thickest.

When the cells were deplasmolysed from stronger solutions there was no apparent swelling of the protoplasm or rupture of the membrane. The cytoplasm layer as a whole appeared to thin out and finally burst. The concentrations of these solutions, however, were much higher than the lowest capable of causing ectoplasmic deplasmolysis injury—especially after one or more hours in the plasmolyte.

These results favour the assumption that the increased deplasmolysis injury resulting from increased time in the plasmolyte is due to a stiffening of the protoplasmic membrane. Table III indicates that this stiffening occurs much more readily in unhardened than in hardened cells.

TABLE III
DEPLASMOLYSIS INJURY TO UNHARDENED AND HARDENED CABBAGE CELLS

Dextrose conc., <i>M</i>	Time in plasmolyte, hr.	Degree of plasmolysis (average of 10 cells)	Cells dead after deplasmolysis, %
(a) Unhardened			
0.5	5½	0.67	75
	6	.63	25
	6½	.66	50
(b) Hardened (9 to 12 days at 5° C.)			
2.0	6	0.28	50
	5½	.27	10
	5	.28	50

A concentration of plasmolyte four times as great was required to cause similar injury to hardened as to unhardened cells. At their respective critical concentrations, the hardened cells were reduced to less than one-third their normal volume, the unhardened to only two-thirds. It is interesting to note that at their critical freezing temperatures the hardened cells are also reduced to less than one-third their normal volume, whereas the unhardened are reduced to one-half. On the other hand, when deplasmolysis injury was determined 15 to 20 min. after immersion in the plasmolyte, the unhardened cells suffered the same injury (about 50%) only when a concentration of 1.5 *M* dextrose was used. In this solution their volume was reduced to less than one-third normal (about the same as the hardened in 2.0 *M* dextrose). The hardened cells, however, were injured to about the same extent whether in the plasmolyte for one-quarter hour or six hours and in both cases were reduced to the same volume as at their critical freezing

temperature. The unhardened cells at their critical freezing temperature were reduced to a volume midway between those that are critical for deplasmolysis injury after one-quarter hour and after six hours in the plasmolyte.

Similar tests were made with hardy and dehardened *Catalpa* twigs. A modification in the technique enabled the cells to round up even in the extremely concentrated solutions that were used for the hardy cells. (It should be mentioned that this method does not work with all plants). Sections of cortex were placed in 3 to 6 cc. of slightly hypotonic dextrose in a watch glass, which was left exposed to the dry atmosphere (about 20% relative humidity) of the laboratory. From time to time as the solution gradually became concentrated, a section was removed and transferred to water. When plasmolysis was sufficiently strong to cause death on transfer to water (usually after about 12 to 15 hours) 10 cells in a similar section were measured (Table IV).

TABLE IV
DEPLASMOLYSIS INJURY IN HARDY AND DEHARDENED
Catalpa CELLS

Dehardened		Hardy	
Degree of plasmolysis	Injury, %	Degree of plasmolysis	Injury, %
0 53	0	0.29	0
.51	50	.28	0
.46	100	.28	0

The hardy cells were not injured even when the solution became supersaturated (about 6 *M*) and their volume was reduced to less than one-third normal; the cells were still alive even two or three days after the test. The dehardened cells were killed when their volume previous to deplasmolysis was reduced to one-half normal. The concentration of solution causing injury to the dehardened was not determined, but on the basis of previous experiments must have been about molar. The osmotic pressure of the supersaturated solution is about 20 times as great as that of a molar solution, and the dehydrating forces are equal to those produced by a freeze of about -20° and -2° C., respectively. Actually, the hardy cells had withstood -20° C. in nature, while an artificial freeze of -5° C. killed the dehardened.

It is interesting to note that the hardy cells were killed if deplasmolysed in water at $+2^{\circ}$ C. (instead of $+20^{\circ}$ to 25° C.) after plasmolysis in supersaturated dextrose at room temperature in the usual way. Furthermore, there was a limit to the length of time the sections could be left in the plasmolyte before deplasmolysis. If this time exceeded about 15 to 20 hours, deplasmolysis from the supersaturated dextrose might cause death. As mentioned above, the dehardened *Catalpa* cells were capable of surviving deplasmolysis from 2 *M* sucrose if placed directly in it and transferred to water a short time (about one-quarter hour) later.

It is evident from these experiments that stiffening of the protoplast surface of non-hardy cells increases greatly with the time in the plasmolyte. In hardy cells there is little change within the times of test. Visual evidence of a difference in non-hardy cells is readily obtained. If sections of de-hardened *Catalpa* or *Hydrangea* were plasmolysed in dextrose solutions, the protoplasts rounded up and, either simultaneously or within some time after rounding up, the protoplasm underwent a distinct change (Plate II, R). The chloroplasts, granules, and granular kinoplasm all accumulated in one clump either at a free end of the protoplast or as a girdle around the middle. The rest of the protoplast was surrounded only by the hyaloplasm. Sometimes the surface of the protoplast was buckled in, owing to tension on a cytoplasm strand. If the concentration of a plasmolyte was too high, these changes might fail to occur. These phenomena were frequent but not constant in unhardened cabbage cells; they were never seen in the hardened.

It is interesting to note that Gessner (4) has observed this "systrophic" balling in the cells of the water leaves of an amphibious *Ranunculus* but not in those of the air leaves. The former proved to be much more sensitive to drought injury, although it is not clear whether this was due to a morphologic or a physiologic difference.

As to the cause of the clumping, Germ (3) considers it a direct result of plasmolytic stimulation and found that it was favoured by any agent that reduced protoplasmic viscosity. This would indicate that after slight plasmolysis has been maintained for some time, the non-hardy cell possesses a lower protoplasmic viscosity than does the hardy.

Correlation with Varietal Hardiness

For practical purposes, deplasmolysis injury is more likely to serve as a test of hardiness if the membrane stiffening is permitted to occur, since the differences seem greater in this case. In the following tests, the sections were therefore left in the plasmolyte for four to five hours before deplasmolysis.

Four alfalfa varieties (two hardy and two tender) were hardened for two weeks at $+5^{\circ}\text{C}$. (with continuous light) after growing for four to five months in the greenhouse. Sections of the cortex were then made from the first half-inch of the root. The osmotic concentration of the cell sap was practically the same in all varieties—about 0.4 *M* calcium chloride (25 atm.).

The sections were cut into isotonic or slightly hypotonic dextrose, evacuated to remove the air from the intercellular spaces, and then transferred to a concentrated solution of dextrose (or invert sugar when more than 3 *M* was used).

Both hardy varieties possessed a distinctly greater resistance to deplasmolysis injury than the more tender varieties (Table V). Control plants (six of each) frozen at -11°C . for seven hours were examined two weeks later. All the Arizona and four of the Kansas plants were dead. Hardistan and Grimm were all alive. In all cases the old foliage was dead.

TABLE V

DEPLASMOLYSIS INJURY TO HARDENED ALFALFA VARIETIES. EACH VALUE FOR AN AVERAGE OF THREE PLANTS GROWN IN THE SAME POT

Variety	Time hardened, days	Conc. of dextrose or invert sugar, M	Injury, %	
Tender	Kansas	11	75	Av. 80
		14	75	
		15	90	
	Arizona	11	100	Av. 90
		14	75	
		15	100	
Hardy	Grimm	11	50	Av. 35
		14	10	
		15	50	
	Hardistan	11	25	Av. 30
		14	0	
		15	70	

From all these experiments certain conclusions may be drawn. The rate of protoplast expansion is obviously a negligible factor in deplasmolysis injury. Nor is the injury dependent solely on the degree of surface extension during deplasmolysis, for cells can be killed as a result of collapse without any change in surface area. This fact and the lack of protoplasmic swelling during deplasmolysis after strong plasmolysis, indicate that the injury may be initiated during dehydration. The damage is obviously not an ion effect, since similar results were obtained with non-dissociating sugars. Yet the injury is dependent not only on the degree of dehydration previous to deplasmolysis, but also on the degree of surface extension during the latter process. In some cases at least, the injury is localized in the outer membrane, the bursting of which is followed by death of the cell. The tendency of this membrane to burst on expansion increases with time in the plasmolyte, owing presumably to increased stiffening. But this occurs much more readily in non-hardy than in hardy cells. The protoplasm of non-hardy cells also undergoes a "systrophic" balling. In the protoplasm of hardy cells this does not occur.

Deplasmolysis injury therefore depends on the physical state and the degree of expansion of the protoplasmic membrane, or in extreme cases, of the protoplasm as a whole.

Ductility of Cytoplasmic Strands

When a protoplast plasmolyses convexly, it still retains cytoplasmic connections with its wall, in the form of fine strands that are usually all but invisible. If its cytoplasm is liquid, its consistency must be high to allow the formation of these strands; if solid, it must be ductile. Observations

of the strands under different conditions should therefore yield some information concerning protoplasmic consistency or ductility.

Cortical cells of *Hydrangea* proved very satisfactory for this study, since they possess somewhat more distinct strands than most other cells, probably owing to the large pits with which the strands are presumably (sometimes visibly) connected. Observations were made on the large cells next to the bark. At least some of these from both hardy and dehardened twigs tended to 'plasmolyse convexly.

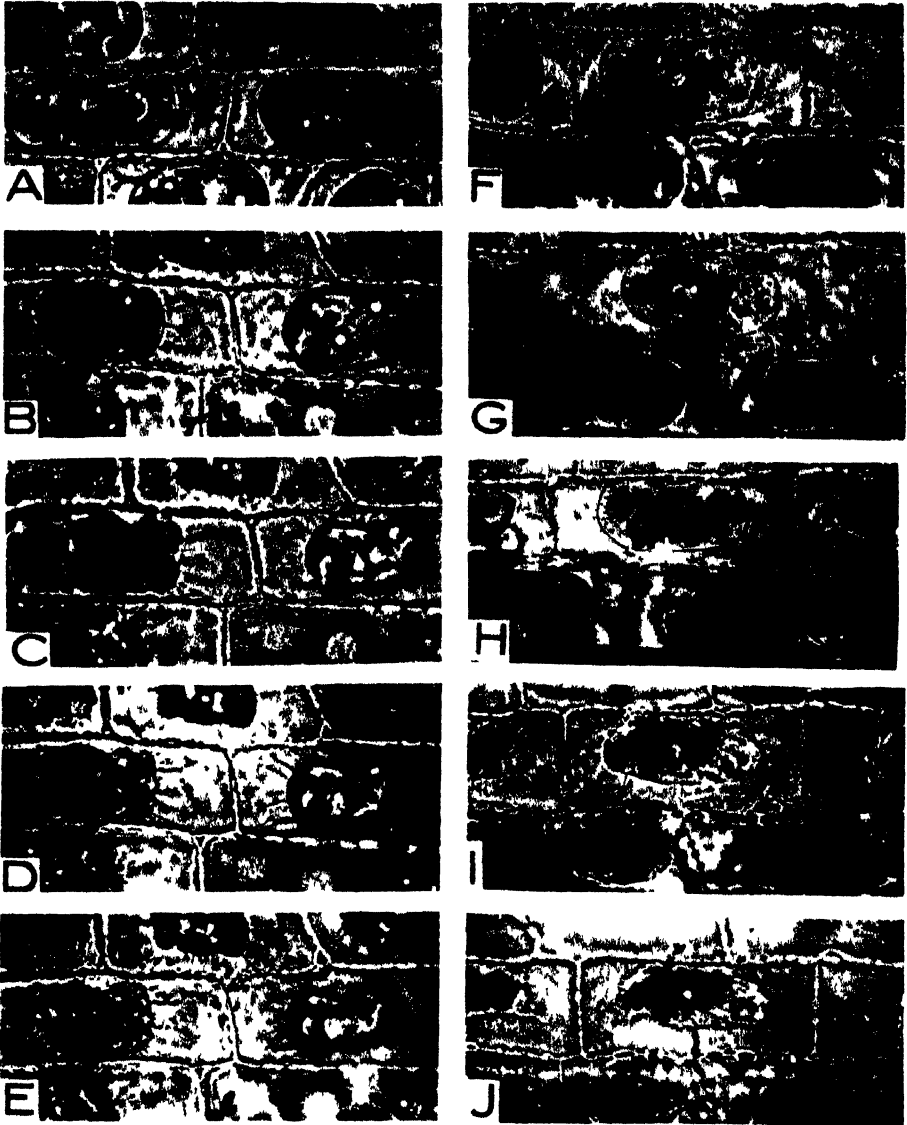
The sections were placed in slightly hypotonic dextrose (0.25 *M* for the dehardened, 0.50 *M* for the hardy) and evacuated to remove the air bubbles in the intercellular spaces. They were then transferred to a hypertonic solution causing shrinkage of the cells to about three-fourths their normal size, and, after about one-half hour, were transferred to a still stronger solution. In this way the same cells were observed in a series of concentrations up to saturated and (in the case of hardy cells) even supersaturated dextrose.

In 0.6 *M* dextrose (and even in solutions of lower concentrations) the dehardened cells showed very fine and barely visible strands (Plate I, *A*). In higher concentrations these became more distinct (Plate I, *B-E*). Since they had been stretched thinner, this could be due only to an increased refractive index resulting from their dehydration. In the case of the hardy cells, on the other hand, the strands were not apparent until a concentration of about 1.5 *M* dextrose was reached (Plate I, *G*). When they did appear they seemed, if anything, thicker than those in the dehardened cells. Consequently the lack of visibility must have been due to a refractive index about identical with that of the surrounding liquid. The obvious conclusion is that the strands of the hardy cells were more highly hydrated than those of the dehardened cells.

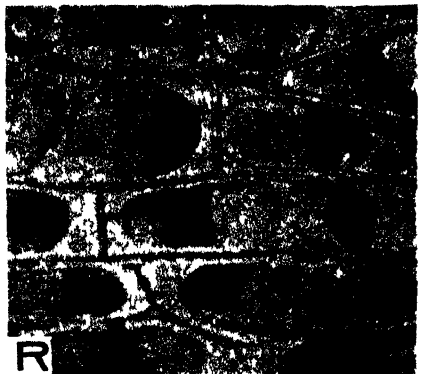
The smooth contours of the protoplasts in the lower concentrations indicate a low consistency. In the higher concentrations, the tension on the strands pulled the surface out, making it irregularly dentate; this occurred in a concentration of 3 *M* in hardy cells and in a lower concentration (1.5 *M*) in the case of the dehardened (Plate I, *I* and *C*). The consistency or plasticity of the dehardened protoplasm obviously rises more rapidly on dehydration than does that of the hardy protoplasm. This is to be expected in view of greater dehydration indicated by the more rapid increase in refractive index. In the highest concentrations (2.5-3 *M*) the strands of the dehardened cells became so rigid and inextensible that some of them actually snapped (Plate I, *E*). This never occurred in the hardy cells, even in much higher concentrations such as supersaturated (6 *M*) dextrose (Plate I, *J*).

In the lowest concentrations, the strands of the dehardened cells frequently showed spherical regions that appeared to be droplets (Plate I, *B* and *C*). This would indicate a low consistency in these concentrations and would again indicate that near their respective isotonic points, dehardened protoplasm has a lower consistency than hardy protoplasm. However, it is also

PLATE I



- A-E. Cortical section of a dehardened *Hydrangea* twig in 0.6, 1.0, 1.5, 2.0, and 3.0 M dextrose, respectively. In A, the section is upside-down relative to the others.
- F-J. Cortical section of a hardy *Hydrangea* twig in 1.0, 1.5, 2.0, 3.0 M and supersaturated (about 6 M) dextrose. In H, the cell at the left of the other photographs is now in the centre.



- K *Dehardened cortical cell of Cornus in M dextrose, injected with an oil drop (Nujol)*
- L *Hardy cortical cells of Cornus in a solution of M sodium chloride + calcium chloride (9:1), each injected with an oil drop (Nujol)*
- M *Dehardened cortical cells of Cornus in M dextrose, each injected with an oil drop (Nujol)*
- N *The same transferred to about 3 M dextrose*
- O *Dehardened cortical cell of Cornus injected with an oil drop (Nujol) in M dextrose, transferred to about 2 M, then deplasmolysed*
- P *Hardy cortical cell of Cornus injected with an oil drop (Nujol) in a solution of M sodium chloride + calcium chloride (9:1), transferred to 4 M, then deplasmolysed (The same result was obtained with dextrose)*
- Q *Cabbage cell with injected oil drop, stretched by outer cytoplasmic surface (the misoplasm can be seen to have collected in the angles between the stretched oil drop and the outer cytoplasmic surface)*
- R *Systrophy in dehardened cortical cells of Catalpa after three hours in 4 M dextrose.*

conceivable that these apparent droplets are merely a type of vacuolation (due perhaps to syneresis) which may frequently be seen in protoplasm (9) and which yields no information concerning the consistency of unaltered protoplasm.

Similar tests with *Catalpa* cells yielded similar results, though the strands were finer and less readily observed. Even the dehardened cells, in which the osmotic pressures were very low and which plasmolysed perfectly convexly, showed strand breakage in the higher concentrations. No breaks were observed in hardy cells.

It may be objected that the strands were pulled out to a greater length in the dehardened than in the hardy cells, since the volume reduction of the protoplast was greater, and that this and not a difference in ductility was the cause of the break. But it appears extremely unlikely that such a slight difference in the extent of stretching has any appreciable effect. Strands from free protoplasts have been pulled out to lengths many times the diameter of the protoplast itself without breaking. Furthermore, it is quite probable that the strand length was as great in hardy cells in 6 *M* dextrose as in dehardened cells in 2.5 *M* dextrose, especially since the protoplast contraction in the final stages occurred more at one side than at the ends.

When plasmolysis is concave, the conditions of the strands may be quite different. Sometimes one or two were seen connecting the concave surface to the wall. In the case of dehardened cells, however, focusing on the upper or lower wall revealed a sort of irregular network. This was never observed in hardy cells. Its significance is not clear, though it may be due to torn strands.

From these results it seems evident that the ectoplasm of non-hardy cells is much more readily dehydrated than that of hardy cells. Its refractive index therefore rises more rapidly, the surface of the protoplast is pulled out more easily by tension on the cytoplasmic strands, which eventually become so brittle that they burst in the case of non-hardy but not in hardy cells.

The Shape of Injected Oil Drops

Chambers and Höfler (1) and Chambers and Kopac (2) have shown that an oil drop released near a protoplast snaps onto it. In the following investigation this fact has been used to study the protoplasmic surface of hardy and non-hardy cells.

A micropipette was forced through a pit in the wall of a plasmolysed cell, and thrust close to the surface of the protoplast. The oil was then ejected from the micropipette onto the protoplast surface. In some cases protoplasts freed from their walls were similarly injected, with essentially the same results. A mineral oil (Nujol) with an interfacial tension of 35 to 40 dynes, and olive oil or oleic acid with a tension of 5 to 10 dynes were used. The tensions were measured both in the dextrose and the balanced sodium chloride and calcium chloride (9 : 1) solutions which served as plasmolytes.

Cortical cells of *Cornus* twigs and parenchyma cells of cabbage petioles were investigated. The former were of two types, "vacuolate" and "avacuolate" (7).

The drop established itself instantaneously at the protoplast surface (i.e., it "penetrated"). The interfacial forces molded it into a lens-shaped body that was either equally or unequally bi-convex or even plano-convex. When the curvature was unequal the stronger convexity was toward the inside of the cell, indenting the vacuole quite obviously when the latter was stained with neutral red. There were no apparent differences in shape, whether hardy or non-hardy cells, "vacuolate" or "avacuolate" cells, or even cells from different kinds of plants were compared (Plate II, K, L).

This behaviour is, of course, true only of small oil drops. With increase in size of the drop, the inner surface changed from convex to plane and finally even to concave if the drop was several times the size of the cell, since surface tension varies with the size of the drop.

Nujol penetrated more easily than olive oil. In fact, only large drops of the latter entered the protoplast at all. When oleic acid penetrated, it dissolved in the protoplasm, producing moribund changes.

In general, ease of penetration of any one oil was similar in different cells plasmolysed to the same degree—even if different kinds of solutions were used (i.e., dextrose or a balanced solution). Strong plasmolysis, using a solution with an osmotic concentration of 100 atm., inhibited the penetration of an oil drop, unless it was several times the size of the contracted protoplast. Thus in the case of dehardened *Cornus* cells plasmolysed in 2 M dextrose, the protoplast snapped onto the oil rather than vice versa. Yet even in the strongest solutions, the volume of hardy *Cornus* cells could not be sufficiently reduced to cause this.

A distinct difference between hardy and non-hardy cells was obtained as follows. After injecting an oil drop into a protoplast immersed in a weak plasmolyte (25 to 50 atm.) the section was transferred to a stronger solution (100 atm. or more). Whether the cells were hardy or not, this transformed the oil drop from a lens to a sphere (Plate II, M, N). If the protoplast was then allowed to deplasmolyse partially, the drop stretched, becoming in some cases flattened and even strongly crescentic. But this reaction depended on the hardness of the cell as well as on the degree of plasmolysis previous to deplasmolysis. Non-hardy *Cornus* or cabbage cells showed marked flattening if the plasmolyte concentration was 50 atm. or more (Plate II, O), hardy cabbage cells if it was 100 atm. or more, whereas hardy *Cornus* cells failed to show any flattening even after plasmolysis in a solution with an osmotic concentration of over 200 atm. (Plate II, P). In the latter case there was merely a reversion from the spherical shape (which resulted from the stronger plasmolysis after injection of the oil drop) to the original lens shape. In the non-hardy cells, on the other hand, the sphere stretched to a crescentic layer even before deplasmolysis reached the stage in which the drop

had originally been lens-shaped. If oil drops were injected into other cells of this same tissue, they assumed a practically normal bi-convex or plano-convex shape, in contrast to the flattened shape in the cells into which oil had been injected before further plasmolysis and deplasmolysis.

After strong plasmolysis, if deplasmolysis was allowed to proceed far enough, non-hardy cells, of course, burst. Just prior to this the oil drop reached its maximum degree of extension. This latter phenomenon, in fact, always permitted prediction of the bursting. As a result of the rupture the oil immediately rounded up owing to the release of the tension.

When a sufficiently thick layer of protoplasm was visible, the stretching of the oil drop was seen to depend solely on the surface membrane (Plate II, Q). The liquid nature of the protoplasm below this membrane was indicated by the active Brownian movement.

Strong plasmolysis obviously caused solidification or gelation of the membrane of non-hardy but not of hardy protoplasts. It is apparently this solidification that is responsible for the deplasmolysis injury and strand rupture noted above.

Discussion

Two facts are fully established: (1) When in equilibrium with the same dehydrating force, the protoplasm of non-hardy cells has a higher consistency than that of hardy cells. (2) In hardy protoplasts maintained in a strongly contracted condition the ectoplasm does not become rigid, whereas in non-hardy protoplasts contracted for the same time and to a considerably lesser degree, the ectoplasm becomes quite rigid.

These two facts can be explained only on the basis of a greater hydrophily in hardy protoplasm; this increase appears to be more striking in the ectoplasm than in the mesoplasm. Consequently, the membrane of hardy protoplasts is more resistant toward dehydrating forces and less easily stiffened than that of non-hardy protoplasts. It is less easily ruptured on subsequent stretching (whether by deplasmolysis or tension on its strands) and has less of a flattening effect on attached oil drops. Its refractive index does not rise so much on dehydration.

Such an increase in hydrophily of the plasma membrane has already been suggested by Scarth and Levitt (7) as the cause of the increased permeability during hardening. The difference is so great that the mesoplasm itself must also be affected (as, indeed, was found to be the case (7)), even if to a lesser degree. This accounts for the differences in the physical properties of the protoplasm as a whole. Yet at moderate degrees of dehydration, when the protoplasm is still fluid, it may conceivably have a higher consistency in hardy cells.

The two facts listed above are also of vital importance from the point of view of frost resistance. They indicate that at any one freezing temperature, the physical properties of protoplasm are much more markedly altered in non-hardy than in hardy cells. The second one points to an interesting

question. Of what importance is the time factor in frost injury? From analogy with deplasmolysis injury, one would expect it to be of prime importance. This point will be considered in a later paper.

Acknowledgments

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EFFECTS OF TALC DUSTS CONTAINING PHYTOHORMONE, NUTRIENT SALTS, AND AN ORGANIC MERCURIAL DISINFECTANT ON THE ROOTING OF DORMANT *TAXUS* CUTTINGS¹

BY N. H. GRACE² AND J. L. FARRAR³

Abstract

Dormant *Taxus cuspidata* cuttings were treated with talc dusts containing 1- and 2- γ -naphthylbutyric acid at concentrations of 0, 500, 1000, and 2000 p.p.m., each taken separately and in combination with 0, 0.1, 1, and 10% of a mixture of nutrient salts and 0 and 50 p.p.m. of ethyl mercuric bromide. Data on the number of rooted cuttings failed to disclose any significant treatment effects. However, the number and length of roots per rooted cutting were increased by the phytohormone in all concentrations, the effect increasing with ascending concentration. Data for most of the other criteria indicated injurious effects from the phytohormone. The average length of new growth was increased by both the 1 and 10% concentrations of nutrient salt in the presence, but not in the absence, of 1000 p.p.m. of the phytohormone. The 10% concentration of nutrient salts decreased the number of cuttings with new growth and the number of such cuttings that were rooted. Organic mercury treatment failed to have any significant effects.

Previous communications have reported on the effects of treating plant cuttings with phytohormones, cane sugar, and organic mercurial disinfectants incorporated in a talc carrier (6, 7). Beneficial effects were obtained by watering the sand in which Norway spruce cuttings were propagated, with a dilute solution of nutrient salts (5). Beneficial and injurious effects were noted when cuttings were soaked in nutrient solutions in the presence and absence of indolylacetic acid (12, 13). In consequence, it was considered of interest to investigate the effects of nutrient salts in dust mixtures. Since the interaction effects of various chemical treatments are often of importance in propagation (6, 7, 11), the nutrient salts were also used in combination with phytohormones and disinfectants. The present communication describes the results of an experiment in which cuttings of *Taxus cuspidata* Sieb. and Zucc. were treated with talc dusts containing a mixture of isomeric naphthylbutyric acids, nutrient salts, and ethyl mercuric bromide. *T. cuspidata* was selected since it is known to root without great difficulty (1, 14, 16, 17).

Experimental

The experiment involved the use of a factorial series of talc dusts containing naphthylbutyric acid at concentrations of 0, 500, 1000, and 2000 p.p.m. (parts of chemical per million parts of talc mixture by weight), each taken separately and in combination with 0, 0.1, 1, and 10% of a mixture of

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nutrient salts and 0 and 50 p.p.m. of ethyl mercuric bromide (4)*. The naphthylbutyric acid was an isomeric mixture of 1- and 2- γ -naphthylbutyric acids†. This chemical has been shown to have physiological activity of about the same order as naphthylacetic acid (9). The individual members of the mixture of nutrient salts and their relative proportions were the same as those of a previously used nutrient solution (5) which, in turn, was based on Hoagland's nutrient solution (15). Master dusts containing phytohormone, nutrient salts, and organic mercury, each separately, were prepared first. Admixture of these and dilution with ball-milled talc permitted the ready preparation of the entire series of 32 dusts.

There were 10 cuttings to a group and three completely randomized replicates of each treatment, providing one level of precision for comparison of all the main effects and their interactions. In addition to the 32 treatments of the factorial series each replicate contained duplicate groups of 10 untreated cuttings. The entire experiment required 1020 cuttings.

Prepared cuttings‡ of 1939 wood, without a heel and ranging from 6 to 8 in. in length, were treated and planted immediately in a relatively coarse brown sand (10). Although it appears likely that better rooting might have been obtained through the use of a peat-sand mixture (10, 11), sand only was chosen, as nutrient effects from the peat might otherwise have masked the action of the nutrient salts added by the dusts. The cuttings, in groups of 10, were sprayed with water prior to dusting to ensure the adhesion of a substantial quantity of dust, and were then planted at a depth of about 1.5 in. (18), on February 15, 1940. The medium was maintained at a temperature of about 72° F. by electrical bottom heat cables. The room temperature ranged around 65° F., the maximum variation being from 50 to 80° F. For the first week after planting, the propagation frame was covered by a factory cotton shade to reduce light intensity and maintain a high relative humidity. The shade was removed for the remainder of the propagation period. The cuttings were watered heavily at planting and thereafter sprinkled and the sand maintained moist. More recent results with Norway spruce (10) suggest that the watering conditions in this experiment were not optimum. The cuttings were removed for examination May 8, 1940.

Record was made of the number of cuttings with new growth, rooted, with new growth and rooted, callused, and surviving. The number of roots was counted and their lengths were measured. Similarly the number and lengths of all new growth shoots were determined. From these observations were calculated the number and lengths of roots per rooted cutting, the mean root

* The ethyl mercuric bromide used in this experiment was prepared by a method developed in the Chemistry Division, National Research Laboratories, Ottawa, by Dr. A. Cambron. This procedure yielded a product consisting of 80% ethyl mercuric bromide and 20% ethyl mercuric chloride.

† The mixture of isomeric naphthylbutyric acids was prepared by Dr. R. H. Manske, Division of Chemistry, National Research Laboratories.

‡ Prepared cuttings were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood.

length, the number and length of new growth shoots per cutting with new growth, and the average length of new growth. All data were analysed by the analysis of variance procedure. Data on numbers of cuttings were subjected to the inverse sine transformation prior to analysis (2).

Results

In Tables I and II are given results of the analyses of variance of the observations. Survival counts were not considered in detail, as very few cuttings with no callus formation survived. For analysis, the number of callused cuttings was combined with the number rooted. In Table I the analyses refer to all the treatments. Table II refers to all treatments except those in which a concentration of 2000 p.p.m. of phytohormone was used, since the meagre data on new growth for this part of the experiment were not suited to the analysis of variance procedure. A number of statistically significant effects from phytohormone and nutrient salt treatment were disclosed by the data. The interactions are not presented in detail in the tables, since, with one exception (Table II), they were all insignificant. Organic mercury failed to have any effect. Data on the significant effects are given in the following tables.

Averaged over the entire experiment, about 27% of the cuttings rooted. About 12% of the untreated cuttings, 17% of the talc treated controls, and 60% of those receiving the best individual treatment were rooted. However,

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF *Taxus*. CUTTINGS TREATED WITH DUSTS CONTAINING NAPHTHYLBUTYRIC ACIDS, NUTRIENT SALTS, AND ETHYL MERCURIC BROMIDE

Source of variance	Degrees of freedom	Mean square						
		Number of cuttings		Number of roots per rooted cutting	Length of root per rooted cutting	Mean root length	Number of cuttings with new growth	Number of rooted cuttings with new growth
		Rooted	Rooted and callused					
Replicates	2	77.0	35.6	101.7	7890	0.09	19.8	32.0
Untreated versus all others	1	667.9	28.6	476.7***	47064*	51.53	254.9	294.3
Treatments:								
Phytohormone	3	416.5	3966.3***	1039.6***	66938***	17.15	4888.9***	945.6***
Nutrient salts	3	243.8	251.8	14.9	642	12.24	327.4*	380.6**
Organic mercury	1	234.4	45.4	0.7	38	16.67	21.1	114.8
Interactions	24	102.0	139.8	35.2	6674	18.16	94.4*	88.0
Error	67	855.7	123.7	41.1	7168	19.91	94.5	80.3

* Exceeds mean square error, 5% level of significance.

** Exceeds mean square error, 1% level of significance.

*** Exceeds mean square error, 0.1% level of significance.

variability in rooting was so great that no differences could be demonstrated statistically. This result emphasizes the importance of designing such experiments in a manner to permit of some test of the significance of the data.

TABLE II
SUPPLEMENTARY ANALYSIS OF VARIANCE OF RESPONSES OF *Taxus* CUTTINGS

Source of variance	Degrees of freedom	Mean square		
		Number of new growth shoots per cutting with new growth	Length of new growth per cutting with new growth	Average length of new growth shoots
Replicates	2	1.06	1375.2*	25.1
Untreated versus all others	1	0.06	165.9	65.7*
Treatments:				
Phytohormone	2	0.39	550.9	228.8***
Nutrient salts	3	0.35	394.8	13.7
Organic mercury	1	0.61	0.2	14.2
Interactions	17	0.18	325.7	26.9*†
Error	51	0.45	400.2	13.0

* Exceeds mean square error, 5% level of significance.

*** Exceeds mean square error, 0.1% level of significance.

† Significance depends entirely on the interaction of phytohormone \times nutrient salts.

TABLE III

AVERAGE RESPONSES OF *Taxus* CUTTINGS ON TREATMENT WITH TALC DUSTS CONTAINING 1- AND 2- γ -NAPHTHYLBUTYRIC ACIDS

Response	Naphthylbutyric acid concentration in talc, p.p.m.				Necessary difference, 5% level
	0	500	1000	2000	
Number of rooted and callused cuttings					
Transformed data	55.3	40.1	35.4	24.3	6.4
Per cent	65.4	42.9	34.6	19.6	
Number of roots per rooted cutting	3.5	29.2	14.8	18.5	3.7
Length of roots per rooted cutting, mm.	35.9	98.4	140.6	153.0	48.8
Number of cuttings with new growth					
Transformed data	51.1	38.5	30.4	17.1	5.6
Per cent	59.6	39.2	26.7	12.1	
Number of rooted cuttings with new growth					
Transformed data	29.8	31.4	28.3	17.5	5.2
Per cent	25.4	28.8	23.3	12.1	
Average length of new growth shoots, mm.	19.0	23.5	24.8	—	2.1

Data on the average effects of phytohormone-nutrient salt treatment are given in Tables III to V. These results demonstrate significant effects from both phytohormone and nutrient salts applied in a carrier dust. The physiological effects of naphthylbutyric acid in all concentrations was brought out most clearly by data on the number and lengths of roots per rooted cutting. The highest concentration, 2000 p.p.m., appeared somewhat too high in some respects, however, as injurious effects were noted, particularly in regard to new growth and percentage of cuttings rooted. Although depression of new growth in *Ribes* cuttings following treatment with the 2000 p.p.m. concentration of indolylbutyric acid has been reported (4), injurious effects from this concentration of the acid on *Taxus*, however, are not in agreement with the findings of other investigators (14).

TABLE IV

AVERAGE RESPONSES OF *Taxus* CUTTINGS ON TREATMENT WITH TALC DUSTS CONTAINING NUTRIENT SALTS

Response	Nutrient salt concentration in talc, %				Necessary difference, 5% level
	0	0.1	1.0	10.0	
Number of cuttings with new growth					
Transformed data	36.4	36.1	35.9	28.8	5.6
Per cent	37.5	37.1	35.8	27.1	
Number of rooted cuttings with new growth					
Transformed data	28.5	28.8	28.9	20.8	5.2
Per cent	25.0	25.4	23.8	15.4	

As was the case in the nutrient watering of Norway spruce cuttings (5), the significant effects of nutrient salt dust treatments related to the new growth (Table IV). It is apparent that 10% is too great a concentration of nutrient salt in the dust, as its effect, on the whole, was injurious. However, in conjunction with the 1000 p.p.m. concentration of phytohormone, even at this level there resulted a substantial increase in the average length of new growth shoots. It may be concluded that there are conditions under which the

TABLE V

INTERACTION EFFECTS OF PHYTOHORMONE AND NUTRIENT SALT TREATMENT ON THE AVERAGE LENGTH OF NEW GROWTH OF *Taxus* CUTTINGS, MM.

Phytohormone concentration in talc, p.p.m.	Nutrient salt concentrations in talc, %			
	0	0.1	1.0	* 10.0
0	19.5	19.3	19.2	17.9
500	25.5	22.3	21.5	24.9
1000	21.9	22.2	28.5	26.8

Necessary difference, 5% level, 3.0.

combination of nutrient salts and phytohormone has beneficial effects in vegetative propagation.

Previous communications have indicated that data on the number and length of roots often provide more information as to the effect of treatment than counts of the number of cuttings rooted (3, 8). The results of this experiment emphasize the importance of this conclusion and also indicate that quantitative data on new growth are of value in indicating physiological activity following treatments.

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ÉTUDES SUR LES HYBRIDES DE CISTES

IV. CORRÉLATION DES CARACTÈRES DU *C. SALVIIFOLIUS* L.

PAR PIERRE DANSEREAU²

Sommaire

Les variétés du *Cistus salviifolius* proposées jusqu'ici par les auteurs ne paraissent pas valides, car l'examen d'un grand nombre d'individus révèle le manque de cohésion des caractères invoqués pour définir ces variétés. Ceci est confirmé par un examen statistique méthodique. Il est démontré, en outre, qu'il n'existe pas de liaisons très nettes des caractères, même considérés deux par deux.

Validité des variétés décrites

On a décrit un grand nombre de variétés du *Cistus salviifolius* L. Dans une monographie du genre (2), l'auteur n'a cru devoir retenir aucune de ces entités, car il lui a paru que la plupart des individus ne correspondaient pas aux descriptions des diverses formes en question. Ainsi, selon Grosser (5), la longueur des pédoncules est censée varier de 2 à 3 cm., puis de 4 à 8 cm.; la longueur des sépales de 7 à 12 mm., puis de 18 à 20 mm. Or, il ne manque pas de plantes ayant des pédoncules de 3 à 4 cm., et des sépales de 12 à 18 mm. Deux autres caractères sont invoqués par Grosser: le nombre des fleurs (un, deux, trois)*, et la présence d'inflorescences latérales. Le premier, de toute nécessité, présente des alternatives sans terme de passage. Pour le second, la plupart des plantes où on le rencontre portent aussi les autres types d'inflorescence, de sorte qu'il n'est pas, lui non plus, absolu.

Chacun des caractères invoqués par Grosser présente donc une variation insensible et n'est susceptible d'aucune répartition en catégories nettes. La seule défense qu'on pourrait faire de ces micromorphes serait leur valeur pratique, leur commodité. Or, il n'en est rien, puisque ces caractères sont rarement liés entre eux, contrairement à ce qu'impliquaient les descriptions jordaniennes de Timbal-Lagrave (9), de Rouy et Foucaud (8), et même celles, plus compréhensives, de Willkomm (10), et de Grosser (5).

Aucun agencement de caractères à l'intérieur du *C. salviifolius* ne présente la même solidité et la même cohésion que ceux qui servent à définir les var. *symphytifolius* et *leucophyllus* du *C. symphytifolius* ou les var. *minor* et *major* du *C. populifolius*, pour prendre des exemples dans le même genre, et où sont pourtant mis en cause des caractères quantitatifs.

¹ Manuscrit original reçu le 3 août 1940 et sous forme révisée le 14 novembre 1940.

² Directeur-adjoint des Services horticoles au Jardin Botanique de Montréal. Chargé de cours à l'Institut Botanique de l'Université de Montréal.

* On rencontre, exceptionnellement, des individus à quatre ou cinq fleurs (P, Fig. 1).

Que la répartition de facteurs quantitatifs soit discontinue dans ces cas et continue chez le *C. salviifolius* peut être l'effet de ségrégations géographiques et d'autres facteurs étrangers au patrimoine héréditaire des espèces. Mais, il n'en demeure pas moins que le résultat atteint nous importe plus que les hypothétiques virtualités de chaque espèce, et que les critères des micro-morphes ne se distinguent de ceux de l'espèce que par l'ordre de grandeur: netteté des caractères, corrélation et fréquence.

Test quantitatif

Après une étude détaillée de plusieurs centaines d'échantillons d'herbier provenant de l'aire entière de l'espèce et de très nombreuses herborisations dans la région méditerranéenne française et italienne, l'auteur a cru pouvoir composer une description complète, de cette espèce polymorphe (2).

Reprenant les caractères variables, il a paru intéressant à l'auteur d'en faire un tableau statistique. C'est par ce moyen, semble-t-il, qu'on peut dessiner le profil biologique d'une entité taxonomique. Qui dit profil, dit contour, limite ou frontière: région de contact avec les entités affines. La statistique permet d'établir la densité d'un caractère et sa résistance aux apports extérieurs dans le cas d'espèces pouvant s'hybrider comme les *Cistus*. Et surtout, la statistique est un test de la validité des entités taxonomiques. Toute révision de groupe, et à fortiori, toute monographie, impliquent une statistique puisqu'elles supposent l'examen d'un matériel plus abondant que celui qui a servi aux descriptions antérieures.

Le test quantitatif envisage l'étendue de la variation des caractères mesurables et le calcul des nombres relatifs d'individus affectés par chaque phase de la variation. De cette tabulation ressortent les traits caractéristiques d'une espèce dans son ensemble, qui ne sont pas observables sur chaque spécimen. Plus un groupe sera homogène et plus grand sera le nombre des individus rencontrés au hasard qui répéteront exactement la formule trouvée pour l'ensemble du groupe. Celle-ci sera extrêmement différente selon qu'on l'appliquera à des espèces fermées (monotypes comme les *Sanguinaria canadensis*, *Tamus communis*, *Dalibarda repens*, *Crambe maritima*), ou à des espèces ouvertes (*Rosa blanda*, *Euphrasia Rostkoviana*, *Rubus orientalis*, *Alchemilla vulgaris*, *Cistus salviifolius*). Autrement dit, l'analyse quantitative ne pourra que refléter le mode de variation propre à un groupe taxonomique donné et qui est ordinairement celui de sa genèse.

En effet, quoi que nous puissions savoir des influences qui agissent sur une espèce donnée, ou même des constituants qui l'ont formée dans le passé, en ce moment actuel de son devenir, il nous faut bien nous la représenter comme la somme des caractères présents chez le plus grand nombre possible des individus qui la concrétisent. Au point de vue biologique, c'est la moyenne, la somme des tendances, qui est le type.

De sorte que, pour chaque espèce, on peut dessiner un profil qui la situe dans le genre et qui exprime le travail de différenciation qui s'y opère dans

son état actuel. Les caractères importants sont les mieux différenciés, les plus nettement spécialisés puisqu'ils servent par excellence à isoler l'espèce, à lui conférer une identité d'autant plus nette qu'elle sera seule à les posséder. Lorsque ces caractères (et le plus grand nombre possible) ont atteint une certaine stabilité (i.e. une présence à un haut pourcentage chez les individus), l'espèce est bien caractérisée. Peut-être approche-t-elle du terme de son évolution et vieillit-elle? Il en va de même à l'intérieur de l'espèce pour les variétés et les formes.

Le test quantitatif, donc, sera particulièrement utile si on l'applique à l'analyse d'espèces encore dynamiques, mal fixées, et plus ou moins insaisissables par les moyens classiques de la taxonomie. Il permettra de déceler les zones de plus grande densité qui existent dans un groupe et de connaître la relation entre l'écart constitué par les extrêmes et le point de concentration du maximum d'individus. Le test quantitatif dégage l'état actuel d'un groupe taxonomique, et sa position précise par rapport aux groupes voisins.

Test qualitatif

Le test qualitatif, d'autre part, recherche des caractères exclusifs ou discontinus de par leur nature même. Ainsi, le nombre de fleurs, un, deux, trois, etc., ne suppose aucun intermédiaire. D'autres cependant, le sont en fait et ceux-là sont intéressants à connaître par la statistique, puisque, à priori, ils appartiennent à des séries qui peuvent comporter des formes de passage. Ainsi les *Solidago canadensis* et *S. lepida* ont des capitules respectivement de 2 à 2.8 mm. et de 3 à 8 mm. de haut; les *Circaea alpina* et *C. latifolia* ont des feuilles respectivement de 3 à 5 cm. et de 5 à 10 cm. de long (7).

Or, la discontinuité des caractères variables est souvent relative; c'est dire qu'elle ne ressort que d'un grand nombre d'observations. Ainsi, le *Populus Tremula* a des feuilles à peu près aussi larges que longues, souvent plus larges que longues, parfois plus longues que larges, tandis que le *P. Tremula* var. *Freyni* a des feuilles toujours plus longues que larges (4). Les deux groupes ont donc une zone de contact, mais cette zone n'implique qu'une faible partie des individus d'un type hétérogène, et n'infirmes en rien la solidité de la variété *Freyni* qui apparaît fort homogène.

La discontinuité peut être observée aussi bien dans une corrélation ou un manque de corrélation entre deux séries de caractères qu'à l'intérieur d'une série d'allélomorphes. Ainsi, les *Oenothera* à étamines égales sont de grandes plantes; les *Cistus* à fleur rouge ont tous un long style, mais un seul *Cistus* à fleur blanche possède ce caractère.

La biométrie permet donc d'établir le coefficient d'instabilité d'un groupe. Ceci peut porter sur le grand nombre des individus et permettre de connaître les extrêmes et la catégorie ou les catégories où se sont formées des concentrations. Appliquée, d'autre part, à l'ontogenèse, et non au seul produit terminal qui sert le plus souvent de base à la définition des groupes, la statistique biométrique permettra une appréciation qualitative des critères spécifiques

d'une très grande valeur. Jentys-Szaferowa (6) a pu ainsi rendre compte du devenir et de la fixation de la forme des feuilles chez les diverses espèces que Linné englobait dans son *Betula alba*: *B. verrucosa*, *B. oycoviensis*, *B. carpatica*, *B. pubescens*. De telles études sont extrêmement précieuses pour la taxonomie, puisqu'elles permettent de toucher et de définir objectivement le processus de la différenciation non seulement dans ses effets mais peut-être aussi dans son action.

En envisageant les problèmes taxonomiques sous cet angle, on pourra se demander sur quels caractères communs il convient de baser les groupes et en vertu de quels écarts, ou de quelles solutions de continuité, il est permis de tracer des frontières. La discontinuité n'est pas forcément le critère final. On peut facilement imaginer une espèce qui ne contienne absolument aucun caractère qui lui soit exclusif, mais une combinaison de caractères exclusive. C'est le cas où le test quantitatif serrera la vérité de plus près que le test qualitatif, plus conventionnel.

Dans la pratique, cependant, les caractères exclusifs sont sans doute les plus intéressants dans un test qualitatif puisqu'ils résultent soit d'une survivance unique ou d'une différenciation très avancée. La fidélité, d'autre part, de certain caractère à tel ou tels autres suppose aussi une fixation, un point terminal dans une voie de différenciation.

Dans le cas qui nous intéresse ici, on peut se demander quel facteur de chaque série est le plus typiquement "*salviifolius*", et ceci indépendamment de sa répartition numérique. C'est-à-dire qu'on peut classer les caractères d'après leur exclusivité à l'espèce en question. Le *C. salviifolius* tient déjà sa consistance de quelques caractères invariables et indissociés. L'un quelconque d'un certain nombre d'autres caractères peut être assimilé à ceux-ci dans la mesure où il partage cette exclusivité, en autant aussi qu'on le trouve associé aux précédents. Le manque d'abondance numérique, s'il y a lieu, ne signifie alors qu'un affaiblissement de la cohésion du complexe "*salviifolius*", ou peut indiquer, au contraire, que le stade actuel est subterminal et que cette cohésion est encore en voie de réalisation, l'espèce étant encore jeune et non stabilisée, mal dégagée encore des influences qui l'ont formée.

Si le pourcentage de coïncidence est très élevé, il ne subsiste alors guère de doute à cet égard et les caractères alternatifs de la même série que le caractère en question n'apparaissent plus que comme des liens, des canaux, si l'on veut, rattachant l'espèce à d'autres espèces (ou rejoignant le phylum primitif du genre?).

Le test qualitatif relève donc la valeur des discontinuités et permet d'apprécier le degré de la différenciation dans le groupe de caractères où elle est encore active. Il se trouvera parfois que cette différenciation sera peu avancée encore pour certains caractères, dans des groupes taxonomiques bien isolés mais dont l'isolement peut être dû surtout à des causes extérieures et non à la structure interne de l'espèce*.

* Ce serait le cas, par exemple, du *Sanguinaria canadensis*, espèce monotype où le nombre des pétales, la forme des feuilles, la longueur des hampes florales, etc. sont mal fixés.

Degré de présence des caractères étudiés chez le *C. salviifolius*

Les spécimens étudiés ici ont presque tous été récoltés depuis moins d'un siècle, espace de temps sans doute assez insignifiant dans la vie d'une espèce aussi largement répandue*.

Les caractères absolus donc sont laissés de côté, puisqu'ils ne manquent dans aucun échantillon étudié et qu'ils figureraient dans les statistiques comme présents à 100%. Ils sont présumés avoir atteint une différenciation définitive et je les appellerai ici caractères primaires: (i) fleur blanche, (ii) cinq sépales, (iii) feuille pétiolée, (iv) style court, (v) cinq loges à l'ovaire, (vi) feuille de moins de 50 mm. de long. Ces six caractères ne se retrouvent jamais ensemble dans une autre espèce: le premier exclut tout le sous-genre *Erythrocistus*; le second exclut les sections *Ladanium* et *Halimoides*; le troisième exclut les *C. monspeliensis* et *C. hirsutus*; le quatrième exclut le *C. varius*, le cinquième le *C. ladaniferus*, et le sixième le *C. populifolius*. Ces caractères primaires suffisent donc à définir le *C. salviifolius*, et toute variation qui ne les atteint pas est insuffisante pour faire sortir l'individu affecté de son orbite spécifique.

Les caractères variables ou secondaires, sont représentés dans la Fig. 1. La procédure suivie a été la même que pour le *C. monspeliensis* (1). Chaque spécimen a été analysé pour cinq séries de caractères représentés par des lettres, de sorte qu'on a pu attribuer à chacun une formule (*CEILN*, *ADIIKN*, etc.). La somme totale de ces formules se trouvera donc exprimer à la fois la fréquence de chaque caractère individuel, leur corrélation entre eux et la distribution géographique des gènes qui les déterminent (Fig. 2).

Trois séries de caractères se rapportent aux feuilles (Fig. 1). D'abord leur dimension: feuilles de plus de 45 mm. de long (*A*), de 25 à 45 mm. (*B*), et de moins de 25 mm. (*C*). On voit (Fig. 2) que les plus petites feuilles (*C*) sont les plus fréquentes, que les grandes feuilles (*A*) sont rares, au plus, et exceptionnellement, 21% en Algérie. Il est à remarquer qu'il ne s'agit ici que des feuilles des rameaux fertiles et non des rameaux stériles et des rejets, dont la forme et les dimensions comme chez bien d'autres espèces (*Platanus*, *Populus*, etc.) varient énormément (4). On peut donc constater la dominance de la microphyllie et supposer que la recombinaison la favorise, étant donnée son abondance actuelle en individus. On peut alors envisager qu'elle résulte d'une meilleure adaptation aux conditions écologiques de l'espèce, ou qu'elle est liée à quelque facteur interne. Ceci est appuyé par le fait que le pourcentage des mésophylles est environ sept fois plus élevé que celui des macrophylls.

Quant à la forme des feuilles, (Fig. 1) elle peut-être: cordée (*D*), ovale (*E*), orbiculaire (*F*), ou allongée (*G*). Les feuilles ovales sont de beaucoup les plus fréquentes étant, en moyenne, et dans presque chaque secteur, plus de deux fois plus nombreuses que chacune des trois autres catégories.

* Il y a une réserve à faire à ce sujet: le milieu où croissent les *C. monspeliensis* et *salviifolius* a encore changé depuis un siècle et a favorisé la dispersion du premier (1, 3).

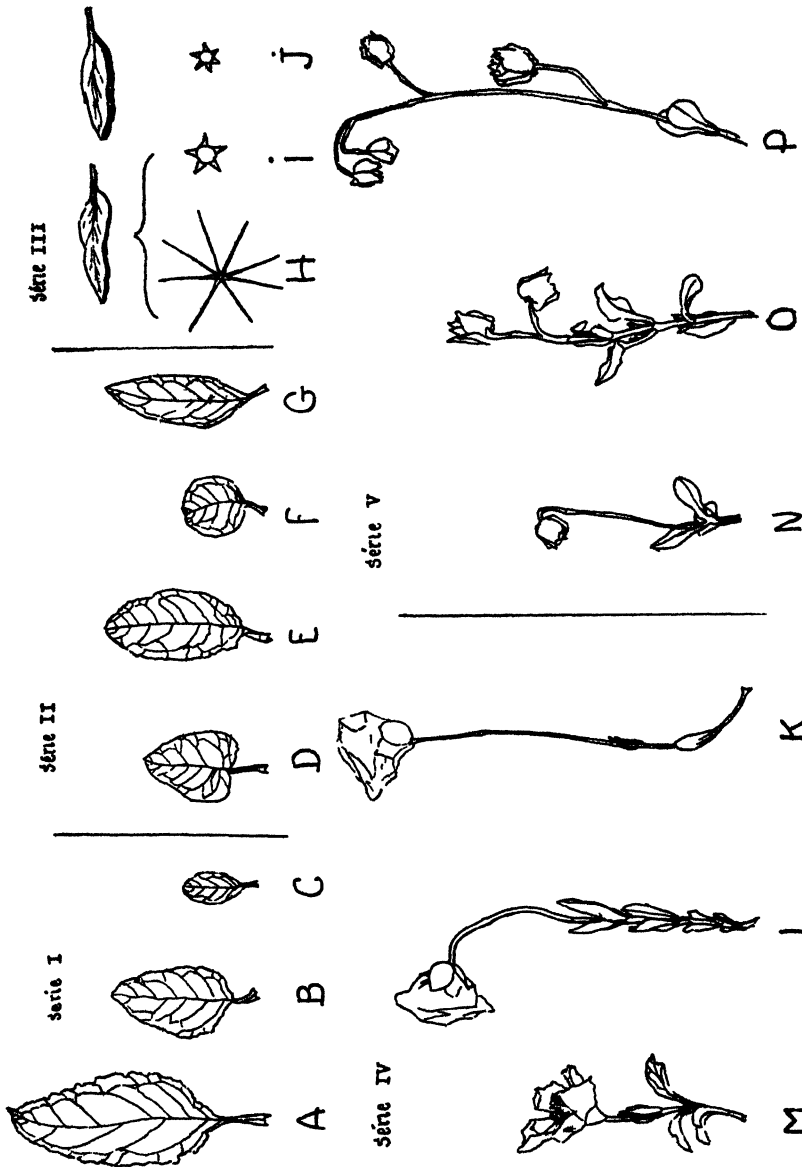


FIG. 1. Caractères du *Cistus subnifolius*. Série I. Longueur de la feuille: (A) 45 mm. et plus, (B) 25 à 45 mm., (C) moins de 25 mm. Série II. Contour de la feuille: (D) cordée, (E) ovale, (F) orbiculaire, (G) allongée. Série III. Indument et texture de la feuille: (H) texture membraneuse et poils étoilés grêles, (I) texture membraneuse et poils étoilés raides, (J) texture spongieuse et poils étoilés raides. Série IV. Longueur de l'inflorescence: (K) 6 à 10 cm., (L) 4 à 6 cm., (M) moins de 4 cm. Série V. Nombre de fleurs: (N) une, (O) deux ou trois, (P) plus de trois.

L'indument des feuilles peut se composer de poils étoilés à branches longues qui donnent à la feuille un aspect tomenteux (H), ou de poils étoilés à branches courtes et rigides formant surface verruqueuse (I); dans ces deux cas, la texture de la feuille est nettement membraneuse; dans un autre cas (J) la texture de la feuille est spongieuse et sa surface nettement verruqueuse. Cette dernière forme est assez peu fréquente. La répartition des deux premières, (H et I) est moins nette, les proportions indiquées ici ne pouvant être prises trop littéralement; car si la moyenne des (H) est de 9% plus élevée que la moyenne des (I), par contre dans six secteurs sur neuf, ce sont les (J) qui

l'emportent. Il faut admettre, en outre, que parfois les feuilles d'un spécimen présentaient un caractère intermédiaire qui rendait le classement difficile sinon un peu arbitraire.

		Lomb. Pié Fr. W. Suisse	Fr. Médit. Italie N.	Esp. et Port.	Italie C. et S. et Sicile	Baléares Corse et Sard.	Grèce, Turq. et Asie Min.	Tunisie	Algérie	Maroc	Moyenne
Série I	A	4	8	4	4	3	4	5	21	5	6
	B	35	46	33	34	14	43	67	43	58	42
	C	61	46	63	62	83	53	28	36	37	52
Série II	D	6	8	15	6	0	10	5	4	9	8
	E	62	50	52	49	47	41	79	45	57	51
	F	13	21	19	27	39	5	3	15	16	19
	G	19	21	14	18	4	44	13	36	18	22
Série III	H	52	41	32	33	28	32	69	31	39	38
	I	42	38	55	54	61	62	20	48	49	49
	J	6	21	13	13	11	6	11	21	12	13
Série IV	K	25	32	16	13	11	11	9	17	23	23
	L	57	41	36	41	25	45	63	54	40	43
	M	18	27	48	46	64	44	28	29	37	34
Série V	N	78	65	44	54	80	40	75	54	70	65
	O	21	33	45	36	6	56	25	46	27	31
	P	1	2	11	10	14	5	0	0	3	4

FIG. 2. Répartition des caractères du *C. salviifolius* exprimée en pourcentage du nombre total des individus examinés et pour chaque région. Les lettres (de A à P) correspondent aux caractères illustrés dans la Fig. 1.

Les deux dernières séries d'observations portent sur l'inflorescence. La longueur peut être de 6 à 10 cm. (K), de 4 à 6 cm. (L), ou de moins de 4 cm. (M). La répartition se présente ici sous la forme d'une courbe régulière, le type moyen étant le plus nombreux et les extrêmes atteignant des proportions élevées. La série est donc quantitativement continue et on peut croire que le jeu des facteurs y est très libre.

Quant au nombre des fleurs, le nombre un (*N*), dépasse du double en moyenne, les nombres deux à trois (*O*), et les individus multiflores (*P*), rarissimes, manquent même totalement à certains secteurs.

La formule *CEILN* correspond à la moyenne la plus élevée pour chaque série de caractères. Or, les caractères *CEIN* sont, dans les séries étudiées, les plus nettement exclusifs au *C. salviifolius*; la longueur d'inflorescence de 40 à 60 mm. (*L*) se trouvant chez toutes les espèces sauf les *C. crispus* et *parviflorus*, notamment chez les espèces affines: *C. hirsutus*, *populifolius*, *monspeliensis*.

Les feuilles plus courtes que 25 mm. (*C*) constituent un caractère exclusif au *C. salviifolius* dans la section *Ledonia*, mais se retrouvent à un haut pourcentage chez le *C. monspeliensis* dans la section *Stephanocarpus* (1). Il faut donc considérer ce facteur comme commun aux deux espèces (puisqu'on le retrouve chez la seconde aux Iles Canaries où manque le *C. salviifolius*). Cette forme ne se retrouve guère dans les autres espèces du genre appartenant à des sections plus éloignées.

La texture verruqueuse des feuilles (*I*) ne reparaît nettement que chez le *C. heterophyllus*, espèce sans liens évidents avec le *C. salviifolius*.

Les inflorescences uniflores (*N*) n'existent que chez les *C. heterophyllus*, *salviifolius*, *ladaniferus*. Malgré l'existence d'hybrides entre ces deux dernières espèces, on ne peut parler d'affinités étroites, vu le nombre et l'importance des caractères qui les séparent, et singulièrement ceux de la fleur et de l'inflorescence.

En résumé donc: le *C. salviifolius* se détache nettement de tous ses congénères par l'association constante de six caractères primaires, accompagnée de la présence fréquente de quatre caractères secondaires (*CEIN*, Fig. 1), plus ou moins exclusifs mais caractéristiques du fait de leur association avec les précédents. Voilà définie la cohésion de l'espèce, sa solidité, sa résistance à l'extérieur*.

L'étude des proportions et des liaisons établies entre les caractères secondaires et leurs alternants nous permettra de nous représenter le dynamisme interne de l'espèce et ce qui lui reste de voies de communication avec d'autres espèces.

Mécanisme de la répartition des caractères chez le *C. salviifolius*

Les écarts à partir des caractères *CEILN* qui sont les plus fréquents, sont dus à plusieurs causes:

- (1) Hétérozygotie de certains allélomorphes;
- (2) Changements écologiques et plasticité relative de l'espèce à cette égard;
- (3) Localisation géographique de certains gènes;
- (4) Introgression de certains facteurs par hybridation avec d'autres espèces, soient les *C. monspeliensis*, *hirsutus*, *populifolius*.

* Le genre *Cistus* est peut-être plus qu'un autre pauvre en gènes, et ses espèces, pour être nettes, n'en résultent pas moins d'un petit nombre de facteurs associés différemment (3).

Il est clair que ces causes peuvent agir conjointement; que la quatrième, par exemple, engendre la première, etc. Quelques cas, cependant, ressortissent à l'une plutôt qu'aux autres.

On ne peut que considérer l'espèce entière comme hétérozygote pour les couples $B-C$, $H-I$, et même $N-O$ (Fig. 2). Ces caractères s'opposent très nettement, et leur présence à un pourcentage très élevé dans chaque secteur, laisse supposer qu'il doit se trouver peu d'individus homozygotes pour l'un ou l'autre des allélomorphes.

Les caractères quantitatifs (longueur des feuilles (A , B , C) et des inflorescences (K , L , M)) subissent forcément l'influence du milieu; on ne sait malheureusement pas si la répartition des plantes à grandes feuilles et à longues inflorescences coïncide toujours avec l'habitat sylvestre et mésophile. La culture, d'autre part, a montré l'influence du sol sur la forme et les dimensions des feuilles. Le diamètre de la fleur varie énormément avec l'exposition et la température (q.v.).

Par contre, la répartition de l'indument des feuilles subit des localisations assez intéressantes (Fig. 2). Les feuilles blanchâtres, veloutées, feutrées (H) se rencontrent surtout au nord de l'aire: région extra-méditerranéenne, nord de l'Italie, sud de la France, Maroc (où la plante atteint une certaine altitude); tandis que la feuille affecte davantage la surface verruqueuse (I) dans les régions où l'habitat xérophytique est plus général (Italie moyenne et méridionale, Espagne, Asie Mineure, Grèce, etc.).

D'autres caractères sont assez nettement hybridogènes. Les feuilles cordées à la base se rencontrent fréquemment en Espagne, dans la France méditerranéenne du sud-ouest, et au Maroc, où l'hybridation avec le *C. populifolius* n'est pas rare. Deux autres caractères sont attribuables à une hybridation plus ou moins ancienne avec le *C. monspeliensis*: la forme allongée des feuilles (G) et un nombre de fleurs supérieur à trois (P). Or, leur localisation géographique respective ne coïncide aucunement. Les individus polyflores (P) sont les plus nombreux dans les îles de la Méditerranée, en Italie et en Espagne; ceux à feuilles allongées (G) en Grèce, en Asie Mineure et en Algérie. Faut-il admettre qu'un même croisement est suivi de recombinaisons différentes à cause de la sélection du milieu?*

Corrélation des caractères du *C. salviifolius*

Le nombre des plantes examinées est 687: 324 combinaisons des 16 caractères étaient possibles. Seulement 151 se sont réalisées. Les caractères C , E , I , L , N , se sont présentés individuellement le plus souvent. Or, la combinaison de cinq caractères la plus fréquente a été $CEIMN$ (41 fois, soit un peu moins de 6%), tandis que la combinaison $CEILN$ est deux fois moins fréquente.

Il devient donc intéressant de rechercher si ces caractères sont de quelque manière liés entre eux, par deux, par trois, par quatre, ou par cinq. On peut

* On peut voir là une tendance, mais une tendance seulement, à la formation d'une variété géographique nouvelle.

considérer les cinq caractères primaires (voir plus haut) comme liés ensemble à 100% et chacun des caractères "secondaires" comme lié à 100% avec eux; tandis que les primaires ne sont liés aux secondaires que dans une proportion toujours très inférieure à 100, mais d'autant plus significative qu'elle s'en rapproche. Un caractère (comme *N*) qui dépasse 50% de fréquence est plus étroitement assimilable aux caractères primaires que les autres et singulièrement que ses alternants (*O* et *P*). De même, un groupe de caractères, atteignant ensemble (par deux, trois, quatre, cinq) un haut pourcentage du total des individus constituerait à l'intérieur de l'espèce un agrégat homogène méritant un rang taxonomique, puisqu'il résulterait d'une spécialisation définissable. Ce rang taxonomique serait naturellement plus élevé s'il s'agissait de cinq que de quatre, de quatre que de trois caractères, etc.

Or, en ce qui concerne les combinaisons de cinq caractères, la corrélation est très faible $CEIMN = 5.96$, $CEHLN = 4.07$, $BEILN = 3.34\%$, etc. Ces chiffres marquent évidemment une absence presque totale de cohésion des caractères considérés par cinq.

Les associations de quatre caractères ne sont guère plus prometteuses. $CEIN$ et $CIMN$ nous donnent 10% du total des individus analysés, $CELN$, 9, $CEMN$, 8.3, $EIMN$, 8%. Ces chiffres sont d'autant moins significatifs que ces cinq combinaisons ont entre elles, prises deux par deux, au moins deux et souvent trois caractères communs.

	I	II	III	IV	V
I	2	4	4	4	4
II	4	2	6	6	5
III	4	6	2	7	5
IV	4	6	7	2	5
V	4	5	5	5	2

FIG. 3. Tableau des corrélations. Les chiffres romains indiquent les numéros des séries de caractères alternants représentées dans la Fig. 1. Les chiffres arabes se rapportent à la figure où la corrélation entre les séries prises deux par deux est relevée.

Sans nous arrêter aux combinaisons de trois facteurs, voyons le tableau de tous les facteurs deux par deux. La Fig. 3 donne le chiffre du tableau où sont relevés les pourcentages d'association de chaque série de caractères (Fig. 1) par rapport à chacune des quatre autres séries. Les figures 4 à 7 donnent donc un tableau complet des associations de caractères. Deux considérations s'imposent ici. Premièrement, existe-t-il deux caractères liés d'une façon exclusive ou presque? Prenons, par exemple l'inflorescence uniflore (*N*) présente chez 65% du total des individus (en moyenne) et la feuille ovale (*E*), présente chez 51% des individus. A priori, la liaison ne

peut être totale, puisqu'elle ne saurait dépasser 51%. Autrement dit, tous les *E* pourraient être liés à des *N*, mais tous les *N* ne sauraient être liés à des *E*, le nombre total de ceux-ci étant inférieur à 65%. En fait, la Fig. 5 nous montre

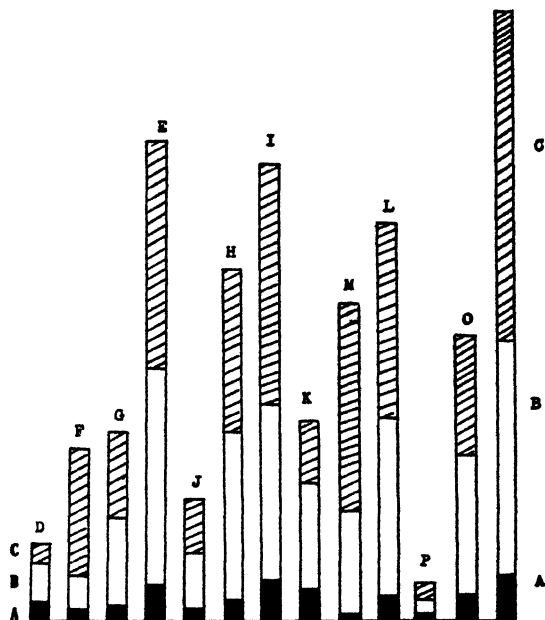


FIG. 4. Corrélation des facteurs (A, B, C) de la première série avec ceux des quatre autres séries.

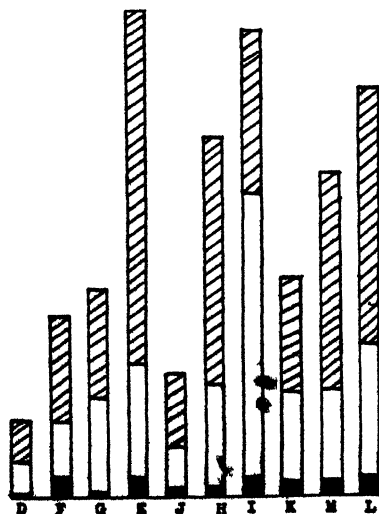


FIG. 5. Corrélation des facteurs (N, O, P) de la cinquième série avec ceux des séries II, III, et IV.

que la liaison *EN* se rencontre chez 37% du nombre total des individus. Ce chiffre correspond à environ 72% des *E* et 57% des *N*. Le reste des *E* (28%) et des *N* (43%) se répartit à peu de chose près proportionnellement au pourcentage moyen du caractère (*D*, *F*, *G*, et *O*, *P*) auquel on les trouve associés.

La Fig. 2 nous avait déjà démontré que nous avions affaire (surtout dans les séries I, II, III, et V) à deux caractères plus ou moins également répartis et à une troisième alternative beaucoup moins fréquente.

Ces derniers, sur les graphiques présentés ici, sont placés en bas. L'on constate qu'ils occupent généralement une longueur proportionnelle à la colonne où ils se trouvent. Les caractères atypiques par excellence donc ne sont ni liés entre eux ni liés à aucun autre caractère, du moins pas dans une proportion significative.

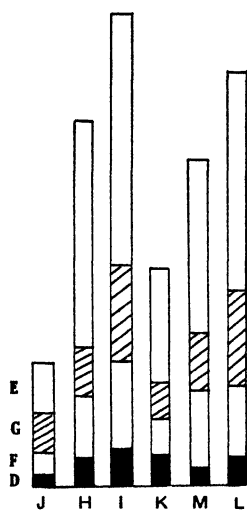


FIG. 6. Corrélation des facteurs (*D*, *E*, *F*, *G*) de la série II avec ceux des séries III et IV.

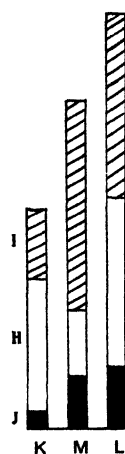


FIG. 7. Corrélation des facteurs des séries III et IV.

La seconde recherche, en l'absence de liaisons absolues, portera sur ce qu'on peut appeler les préférences. On peut classer ainsi une association atteignant un haut pourcentage du total ou dépassant de beaucoup les pourcentages atteints par toutes les autres combinaisons de deux facteurs des deux mêmes séries, ou encore toute association de deux caractères non proportionnelle à leurs pourcentages respectifs.

Dans le premier cas, on arrive à définir plus exactement le type que par les seuls relevés de la Fig. 2; et dans le second cas, on peut discerner des tendances sinon un commencement de ségrégation.

Le *C. salviifolius* sera donc typique quand il possédera les caractères: (i) feuilles petites (*C*) ou moyennes (*B*), (ii) ovales (*E*), (iii) blanchâtres (*H*) ou verruqueuses (*I*), (iv) l'inflorescence moyenne (*L*) ou longue (*K*), (v) uniflore (*N*). La seule liaison qui mérite d'être appelée typique est la liaison *EN*.

Les autres caractères ne manifestent que des tendances: (i) les feuilles grandes (*A*), (ii) cordées (*D*), (iii) subcharnues (*J*), (iv) les inflorescences courtes (*M*), (v) biflores (*O*) ou multiflores (*P*). Pour les caractères des troisième et cinquième séries, deux tendances opposées se manifestent: inflorescence uniflore, feuilles blanchâtres ($HN = 25\%$ du total), et inflorescences à deux ou trois fleurs, feuilles verruqueuses ($IO = 30\%$ du total). Le facteur inflorescence courte (*M*) semble, lui aussi, se lier plus que ses alternants (*L* et *K*) au facteur feuille grande (*A*) i.e., 3.6% du total, soit presque 50% des (*A*).

Aucune de ces tendances, si intéressantes soient-elles au point de vue biologique, ne mérite, à mon avis, d'être reconnue comme entité taxonomique distincte. Il est particulièrement à noter que les caractères si souvent invoqués de l'inflorescence plus ou moins longue (*K*, *L*, *M*) et surtout biflore à multiflore (*O*, *P*) ne sont liés à aucun autre d'une façon constante.

Conclusions

Le *Cistus salviifolius* est une espèce très polymorphe comme le prouve la répartition numérique de plusieurs séries de caractères.

D'autre part, la répartition de ces caractères chez les individus prouve qu'ils ne manifestent entre eux qu'une cohésion négligeable: il devient à peu près impossible de décrire des micromorphes basés sur plus d'un caractère.

L'hybridation intraspécifique est donc supposée intense, d'autant plus que les Cistes sont généralement autostériles. Cette variation continue est accentuée par l'action du milieu.

L'hybridation avec d'autres espèces (les *C. monspeliensis*, *hirsutus*, et *populifolius*, surtout) permet l'introduction, dans un complexe déjà chargé, de caractères nouveaux (*G*, *P*) ou l'accentuation de caractères déjà existants.

Le présent essai n'est pas une étude biométrique, car le matériel étudié est forcément hétérogène (provenant de toute l'aire de l'espèce) et des chiffres trop précis n'auraient que très peu de valeur. L'auteur a seulement voulu démontrer la grande liberté du jeu des facteurs. Ce cas, dans le genre *Cistus* est extrême; les autres espèces suivent des voies de différenciation identifiables, tandis que le *C. salviifolius* est indifférencié par rapport à plusieurs groupes de caractères.

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EFFECTS OF TALC AND PHYTOHORMONE TREATMENT ON THE ROOTING OF *DAHLIA* CUTTINGS¹

BY N. H. GRACE²

Abstract

Groups of *Dahlia* cuttings, untreated, talc treated, and treated with talc containing various concentrations of naphthylbutyric acid, were propagated in sand in a greenhouse. All the untreated cuttings died; those treated with talc alone suffered only 4% mortality. Although phytohormone treatment increased the number of roots per rooted cutting, it increased the average mortality to 23%. There were no significant differences in the effects of the various concentrations of phytohormone. Reduction of mortality by talc treatment was the chief feature of the results.

It is well known that *Dahlia* varieties may be propagated by means of stem cuttings (6). The manufacturers of several phytohormone preparations recommend the use of their products, at definite concentrations, for the successful propagation of *Dahlia* by this means; similar recommendations occur in the literature (5). However, the value of phytohormone treatments in the case of this plant has been questioned, in conversation, by several commercial propagators. This communication reports the results of an experiment in which *Dahlia* cuttings were treated with naphthylbutyric acid in a series of concentrations, in a talc carrier.

Experimental

The experiment involved the use of a group of untreated cuttings, a group dusted with talc only, and groups receiving dust treatments with 1- and 2- γ -naphthylbutyric acid at concentrations of 250, 500, 1000, and 2000 p.p.m. (parts of chemical to a million parts of the talc mixture by weight) (2, 4). There were five randomized replicates of the foregoing six treatments, with five cuttings to the group, 150 cuttings thus being required. *Dahlia* (variety Sagamore Gold) cuttings were taken from the plants on April 12, 1940, approximately four weeks after the tubers had been planted. The groups of five cuttings were weighed, dusted, and planted immediately in a relatively coarse brown builders' sand (3). For the first week after planting the propagation frame was covered with a factory cotton screen. The frame was not provided with bottom heat. The temperature of the greenhouse approximated 65° F., though it frequently rose to 75° F. during the day.

Cuttings were removed about five weeks after planting and records were taken of the number of cuttings dead, rooted, the number and lengths of root, the fresh root weight, and the green weight of the living cuttings. The numbers and lengths of roots per rooted cutting and the mean root length were calculated. Data on the numbers of cuttings were subjected to the inverse sine transformation prior to statistical analysis (1).

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Results

The chief feature of the results relates to the effects of talc treatment. All cuttings with no treatment died within one week of planting. In consequence, the analyses of variance of the responses of *Dahlia* cuttings were confined to the five groups receiving treatment with talc only and talc containing naphthylbutyric acid. The results of the analyses of variance are given in Table I. It is apparent that statistically significant treatment effects were shown only by the number of cuttings dead and the number of roots per rooted cutting. Mortality following naphthylbutyric acid treatment was 23% and after talc treatment alone, 4%; the number of roots per rooted cutting was 3.7 and 1.5, respectively. Whereas phytohormone treatment increased both the number of roots and mortality, there were no statistically significant differences attributable to varying concentrations. Rooting, averaged over the experiment, was 48%. Although the data on rooting of the cuttings were too variable to demonstrate significant treatment effects, it may be mentioned that the proportion of cuttings rooted was: 36% of those talc treated, 51% of all cuttings treated with phytohormone in talc, and 60% of those receiving the best phytohormone-talc treatment.

TABLE I
ANALYSIS OF VARIANCE OF RESPONSES OF *Dahlia* CUTTINGS TREATED WITH NAPHTHYL-BUTYRIC ACIDS IN TALC

Source of variance	Degrees of freedom	Mean square						
		No. of cuttings		No. of roots per rooted cutting	Length of roots per rooted cutting	Mean root length	Fresh root weight	Final green weight of cuttings
		Rooted	Dead					
Replicates	4	606 0	656 5*	3 30	17936	214 5	197 0	26 06
Treatments	4	417 4	355 2	9 78	9518	221 2	315 5	4 66
Talc vs. others	1	978 4	1260 3*	19 18*	6691	388 1	531 8	7 84
Error	16	344 7	193 5	4 06	10158	240 6	249 5	31.84

* Exceeds mean square error, 5% level of significance.

These results demonstrate that talc treatment is markedly beneficial in reducing mortality of *Dahlia* cuttings. The addition of phytohormone results in a marked increase in the number of roots formed, but tends to increase mortality.

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SOME FACTORS AFFECTING THE VIRULENCE OF ARTIFICIAL INOCULUM OF *HELMINTHOSPORIUM SATIVUM* P. K. AND B. AND OF *FUSARIUM CULMORUM* (W. G. SM.) SACC.¹

BY L. E. TYNER²

Abstract

Investigations were conducted on the virulence of inoculum of *Helminthosporium sativum* P. K. and B. and of *Fusarium culmorum* (W. G. Sm.) Sacc. as affected by the size of the vessel in which the inoculum was increased, the amount of corn meal present in the medium, and the period of incubation. Inoculum of either pathogen containing 12% corn meal caused more disease on wheat seedlings than that with 5%. *H. sativum*, 14 days old, was more virulent than after 21, 28, or 35 days' incubation, but in the case of *F. culmorum*, there was no definite tendency with respect to the effect of age. The size of container was unimportant if desiccation was avoided.

Introduction

Methods of producing artificial epidemics of air-borne diseases are quite well standardized, because the importance of etiological conditions is appreciated. The method usually employed is to apply a determinate spore load, either dry or suspended in aqueous solution. In the case of soil-borne diseases, it is the practice to apply the inoculum either to the soil in which the plants are grown, or to the seed itself. When it is added to the soil, a carrier such as coarsely ground oat hulls or soil, with various organic admixtures, is essential. Too frequently the effect of these adjuncts is overlooked in the production of the inoculum, with the result that inconsistent data are secured.

Sallans (2), reviewed the methods used by others to inoculate wheat with *Helminthosporium sativum* P. K. and B. and reported his own results. As inocula he used spore suspensions and spores borne in an oat hull medium. Infection was found to decrease with increase in age of the oat hull inoculum and also with curtailment of amount applied. An adverse effect of the carbohydrates in the carrier was noted in the controls, therefore he recommended the use of seed soaked in a suspension of the pathogen for extensive experiments.

Inasmuch as work at this laboratory has proved that soil combined with a minimum of corn meal is a satisfactory medium in which to increase *Helminthosporium sativum* P. K. and B. and *Fusarium culmorum* (W. G. Sm.) Sacc. for root-rot studies, information concerning the effect on virulence of certain factors, including size of the vessel in which the inoculum was increased, the amount of corn meal present, and the age of the inoculum was important.

Materials and Methods

Black loam containing 20% water, was mixed with 5 or 12% corn meal by weight, as required. The media were steam sterilized in 150 and 600

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gm. quantities in 200 and 1000 ml. Erlenmeyer flasks, respectively. When cool, a number of these flasks were seeded with aqueous spore suspension of *H. sativum* or *F. culmorum* and incubated at room temperature. Corresponding flasks were seeded at subsequent intervals to provide inocula of required ages.

The spore suspensions were added aseptically in amounts of 0.5 and 0.3 ml. to the large and small flasks, respectively. The suspensions were made by adding 10 ml. of sterile distilled water to a standard size test tube containing *H. sativum* or *F. culmorum*, cultured on slants of potato dextrose agar for two weeks.

The tests for virulence were made under greenhouse conditions in 6-in. pots. In the first and second experiments there were two series, namely, one with steam sterilized soil, and another with unsterilized soil. The sterilized soil series was omitted in the third experiment. Twenty surface-disinfected, uniform, Marquis wheat seeds were planted in four replicates and the inocula applied at seed level. Thirty grams of inoculum was used in the first and second experiments and 20 gm. in the third.

Notes on height and dry weight of the plants and degree of infection were taken after 40 days. The values obtained were weighted in order to compensate for the differences in numbers of surviving plants. For dry weight data, the actual weight of surviving plants was divided by the number of plants surviving in corresponding control pots. Infection data were weighted by allowing a rating of 100% on plants not surviving, either by failure to emerge or by death during growth. The standard was the number of plants surviving in the controls.

Results

Experiment I

This experiment was carried out during January and February, 1940. Inoculum of *H. sativum* only was employed, at the rate of 30 gm. per pot. Equivalent amounts of unseeded soil corn meal mixture were used for the control pots. One lot of inoculum had been incubated for 14 days and another for 28 days. The data from this experiment are presented in Table I, and also statistical analysis of the data, according to the Analysis of Variance method of Fisher (1), in Table IV.

The seedlings in both sterilized and unsterilized soil series were severely diseased, but the damage was greater in the former. It was found that in both sterilized and unsterilized soil series, the amount of corn meal in the inoculum, the size of flask used to incubate it, and the period of incubation caused significant differences in the number of surviving plants, weighted infection ratings, and weighted dry weights. The inoculum containing 12% corn meal caused more disease than that containing 5%. Also, the inoculum incubated in litre flasks, in 600 gm. quantities, caused slightly more disease than that incubated in 200 ml. flasks. Moreover, the inoculum incubated for 14 days was considerably more pathogenic than that incubated for 28 days. However, with regard to the observed infection rating and the actual dry

weight data, significant differences appeared only in the case of inocula of different ages.

TABLE I

EFFECT OF AMOUNT OF CORN MEAL, SIZE OF CONTAINER, AND PERIOD OF INCUBATION ON THE VIRULENCE OF ARTIFICIAL INOCULUM OF *Helminthosporium sativum* ON WHEAT SEEDLINGS. GREENHOUSE, JANUARY AND FEBRUARY, 1940.

Corn meal, %	Flask, ml.	Incubation, days	Number surviving plants, av.	Average infection, %		Average dry weight, mg.	
				Observed	Weighted	Observed	Weighted
(A) Sterilized soil							
5	1000	14	4.5	60.6	92.7	23.3	5.7
5	1000	28	11.5	70.2	81.7	29.1	17.5
5	200	14	10.2	75.7	86.5	29.3	16
5	200	28	13	63.1	74	32.9	23
12	1000	14	2.7	80.9	97	20	3
12	1000	28	5.2	76.2	93.2	21.8	6
12	200	14	6.2	78.8	92.7	22	7.2
12	200	28	9	56.7	83	34.7	16.7
5	Control	Control	18.7	3.9	3.9	71.3	71.3
12	Control	Control	18.5	3.8	3.8	67.1	67.1
(B) Unsterilized soil							
5	1000	14	4.5	61.1	91	45.6	10.5
5	1000	28	9.7	48.7	69.7	45.8	23.5
5	200	14	9.2	53.1	77	36.8	18
5	200	28	12.7	31.2	52.5	46.9	31.5
12	1000	14	4.7	71	92.7	25.8	6.2
12	1000	28	5.7	56.5	87	38.7	13.5
12	200	14	6.5	78.8	91.7	27.7	9.7
12	200	28	5.5	47.3	84.7	44.1	12.7
5	Control	Control	19.2	5.8	5.8	60.3	60.3
12	Control	Control	18.7	5.9	5.9	53.3	53.3

Experiment II

Plantings were made in April and both *H. sativum* and *F. culmorum* were used as pathogens. In order to prevent harmful loss of moisture from the media, waxed paper caps were fitted on all flasks after sterilization. The inoculum was incubated for 14 and 35 days. Thirty grams of it was applied per pot in the steam sterilized and unsterilized soil series. The data are shown in Tables II and IV.

Severe infection resulted from both *H. sativum* and *F. culmorum*. There were no significant differences in disease severity between the various treatments in the steam sterilized and unsterilized soil in the *H. sativum* series. However, it is possible that the general severity of the disease may have masked differences which would have been noticeable with less disease.

In the *F. culmorum* series, with few exceptions, the amount of corn meal in the inoculum, and the period of incubation caused significant differences in number of surviving plants, observed and weighted infection, and the observed and weighted dry weights. Inoculum containing 12% corn meal

TABLE II

EFFECT OF CONTENT OF CORN MEAL, SIZE OF CONTAINER, AND PERIOD OF INCUBATION ON THE VIRULENCE OF ARTIFICIAL INOCULUM OF *Helminthosporium sativum* AND *Fusarium culmorum* ON WHEAT SEEDLINGS. GREENHOUSE, APRIL AND MAY, 1940.

Corn meal, %	Flask, ml.	Incubation, days	Number surviving plants, av.	Average infection, %		Average dry weight, mg.	
				Observed	Weighted	Observed	Weighted

(A) *H. sativum*, sterilized soil

5	1000	14	9	55.5	77.7	43.5	20.5
5	1000	35	6.5	50	85.2	44.7	15.5
5	200	14	9.2	60.6	78.2	52.2	26
5	200	35	10.5	50.9	71.5	44.5	26.2
12	1000	14	10.5	61.7	81	34.5	19.5
12	1000	35	5.7	48.7	84.2	49.5	10.7
12	200	14	3.7	81.3	96	26.7	5.2
12	200	35	9.2	35.7	67.7	58	28.7
5	Control	Control	18	5.3	5.3	64.2	64.2
12	Control	Control	18.7	14.4	14.4	75	75

(B) *H. sativum*, unsterilized soil

5	1000	14	12.7	36.1	49.2	86	68.2
5	1000	35	6.5	32.3	72.7	100	39.7
5	200	14	7	38.8	73	80.2	35.7
5	200	35	6	30.4	73.7	64.7	33
12	1000	14	10.2	28.3	53.7	70.2	46
12	1000	35	8	33.3	66.7	71.5	36.5
12	200	14	5.7	50	81.7	79.5	29
12	200	35	11.2	20	43.2	86.2	59.2
5	Control	Control	16	36.7	36.7	80	80
12	Control	Control	16	25.8	25.8	59.2	59.2

(C) *F. culmorum*, sterilized soil

5	1000	14	5.7	57	85	28	8.5
5	1000	35	6	42.9	89.5	28.2	8.7
5	200	14	7.5	46.7	77.7	30.5	13.7
5	200	35	5.2	56.3	87	19.2	5.2
12	1000	14	4.7	73.7	86	16.2	4.7
12	1000	35	2.7	49.1	94.2	18.7	3
12	200	14	3.5	54.3	91.2	42	7.5
12	200	35	2.2	84.4	97.7	20	2.2
5	Control	Control	18	5.3	5.3	64.2	64.2
12	Control	Control	18.7	14.4	14.4	75	75

(D) *F. culmorum*, unsterilized soil

5	1000	14	8.7	27.4	60.2	50.2	27.7
5	1000	35	4.2	39.4	81.2	31.7	9
5	200	14	8	18.4	59.2	44.5	21.5
5	200	35	6.2	35.3	74.7	43	15.7
12	1000	14	2.7	20	86	41.2	6.7
12	1000	35	3.2	68.5	93.5	20	3.7
12	200	14	5.7	18.7	73.3	34	15
12	200	35	3.5	45	87.7	28.2	6.5
5	Control	Control	16	36.7	36.7	80	80
12	Control	Control	16	25.8	25.8	59.2	59.2

was more pathogenic than that containing 5%. The *F. culmorum* inoculum of five-weeks' incubation caused more disease than that incubated for two weeks. The size of container in which the inoculum was incubated had no significant effect on virulence in this experiment.

Experiment III

This test was undertaken to determine whether capping of the flasks during incubation prevented the observed differences in virulence of inocula increased in 1000 and 200 ml. flasks, and to determine also whether possible differences

TABLE III

EFFECT OF CONTENT OF CORN MEAL, SIZE OF CONTAINER, AND PERIOD OF INCUBATION ON THE VIRULENCE OF ARTIFICIAL INOCULUM OF *Helminthosporium sativum* AND *Fusarium culmorum* ON WHEAT SEEDLINGS. GREENHOUSE, JUNE AND JULY, 1940.

Corn meal, %	Flask, ml.	Incubation, days	Number surviving plants, av.	Average infection, %		Average dry weight, mg.	
				Observed	Weighted	Observed	Weighted
(A) <i>H. sativum</i>							
5	1000	14	8.2	48.7	75	118	75.5
5	200	14	9.7	45.5	67	114.2	68.7
12	1000	14	7	52.7	79	73.2	29.2
12	200	14	9.2	43.7	67.5	97.5	57.2
5	1000	21	13	26	41.5	114.7	90.2
5	200	21	15.2	40	45	107	100
12	1000	21	11.2	49.2	64.2	78.7	56
12	200	21	9	44.2	67.7	122.7	68.7
5	1000	28	15	44	49.5	91	82.2
5	200	28	15.5	47.2	50.7	81.2	76.5
12	1000	28	5.7	44.2	79.2	49.2	17.7
12	200	28	4.7	38.7	82.7	85	18.2
5	1000	35	15	33	38.7	68.5	61
5	200	35	18.2	27.5	27.5	103.5	103.5
12	1000	35	13	34.2	47.2	77.7	62.2
12	200	35	5.5	26.7	74.2	89	35
(B) <i>F. culmorum</i>							
5	1000	14	12.7	22.2	39.5	71.5	53.5
5	200	14	12.7	28	44.5	62	46.2
12	1000	14	11.5	39.7	55.5	53	37.7
12	200	14	9.2	19.2	50	85.5	50.7
5	1000	21	13.2	17	33.5	88.7	70.7
5	200	21	14.7	18.7	28.5	67	60.5
12	1000	21	12.5	23.2	37.7	57.2	45.7
12	200	21	9.7	28.2	54	81.5	51.7
5	1000	28	14.5	22.7	32.7	84.5	70.5
5	200	28	13.7	15.2	28	80.2	67.5
12	1000	28	15.2	18	24	98	96.5
12	200	28	11.7	24.7	43.7	75.2	61.2
5	1000	35	15.2	25.5	31.7	63.2	57.2
5	200	35	15.5	22.2	26.7	54.7	51.5
12	1000	35	11	30.7	51	40.5	28.5
12	200	35	11.7	32.5	49.7	63.2	46
5	Control	Control	16.5	8	8	102	102
12	Control	Control	15.5	9.5	9.5	88	88
12	Control	Control	17.4	8.4	8.4	92.6	92.6

TABLE IV
F. VALUES* OF DATA FROM TABLES I, II, AND III

Variable	Number surviving plants	Average infection		Average dry weight	
		Observed	Weighted	Observed	Weighted
<i>Table I, A</i>					
Amount corn meal	22.53	3.97	13.28	.05	16.88
Size container	18.53	1.53	11.22	1.78	18.65
Days incubated	19.80	5.79	18.92	17.65	19.26
<i>Table I, B</i>					
Amount corn meal	22.94	30.28	25.91	3.12	30.11
Size container	7.88	1.64	7.08	.05	5.67
Days incubated	8.84	35.45	20.35	4.75	15.48
<i>Table II, A</i>					
Amount corn meal	1.79	.00	1.36	.55	3.22
Size container	.00	.00	1.12	.18	2.23
Days incubated	.00	9.69	3.03	3.31	.54
<i>Table II, B</i>					
Amount corn meal	.40	.08	.86	3.30	.04
Size container	2.50	.08	1.35	.44	.12
Days incubated	.71	6.95	.00	1.78	1.19
<i>Table II, C</i>					
Amount corn meal	24.63	5.58	10.81	.27	10.07
Size container	.01	.79	.00	1.39	.45
Days incubated	4.98	1.00	9.76	3.08	6.70
<i>Table II, D</i>					
Amount corn meal	16.43	2.80	21.42	4.87	16.65
Size container	2.31	2.53	3.25	.09	1.24
Days incubated	7.30	24.70	16.80	5.19	12.23
<i>Table III, A</i>					
Amount corn meal	36.85	1.27	52.80	4.88	54.35
Size container	.00	.89	.13	5.20	1.61
Days incubated	4.56	9.21	15.20	3.80	5.74
<i>Table III, B</i>					
Amount corn meal	21.89	6.42	17.95	.12	2.28
Size container	2.48	.34	.67	.07	.40
Days incubated	3.54	3.02	4.46	39.30	6.96

* Tables I and II. 5% point, 4.17; 1% point, 7.56.

Table III. Amount corn meal: 5% point, 4.00; 1% point, 7.08.

Size container: 5% point, 4.00; 1% point, 7.08.

Days incubated: 5% point, 2.76; 1% point, 4.13.

from the various treatments might become evident under less severe infection than occurred in the *H. sativum* series of Experiment II.

The flasks were again capped with waxed paper in order to eliminate the desiccation factor. Also, to diminish the degree of infection, only 20 gm. of inoculum per pot was used. The inoculum was incubated for periods of 14, 21, 28, and 35 days to give a greater range of the age factor. In this experiment, only unsterilized soil was used in the pots. Two kinds of controls were employed, namely, pots with unseeded medium plus unsterilized soil, and

those with unsterilized soil only. These two types of controls were expected to indicate the adverse effect of added carbohydrate material. The results appear in Tables III and IV.

In the *H. sativum* series, significant differences for effects of varying amounts of corn meal and of various periods of incubation were found in almost all cases. The inoculum containing 12% corn meal was, as in previous experiments, more pathogenic than that containing 5%. The 14-day-old inoculum caused more disease than that 35 days old, whereas the results from the inoculum incubated for 21 and 28 days were intermediate.

In the *F. culmorum* series, significant differences were found in almost all cases for the effects of the amount of corn meal added and the period of incubation. The inoculum containing 12% corn meal was more pathogenic than that with 5%. The inocula incubated for 14 and 35 days were about equally pathogenic and somewhat exceeded those in the 21- and 28-day series in causing disease. The size of container did not affect the virulence of the inoculum.

Discussion

The results presented emphasize the importance of standardized technique in the preparation of media in which to increase certain soil-inhabiting pathogens. The amount of organic matter incorporated in the medium affects the virulence of inoculum of *H. sativum* and *F. culmorum*. If soil is used as a carrier, the data presented here indicate that in order to prevent organic matter from causing increased virulence, the amount of carbohydrate added should be a minimum commensurate with good growth of the pathogen.

Also, under certain conditions, differences of only one week in the period of incubation of the inocula of *H. sativum* and *F. culmorum* may noticeably affect their virulence; greater differences in age would be expected to produce more marked effects, as Sallans (2) has shown for oat hull medium.

Further, the virulence of the inoculum may be affected by the amount of medium seeded to the pathogen, if the physical conditions of storage are such that irregularities in the rate of water loss, and in air humidity within the flasks, occur as a result of larger differences in the volume of container or medium. Also, excessive desiccation of inoculum can occur, especially in small flasks, under conditions existing in most laboratories; control of this factor is advisable.

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STUDIES ON BACTERIOPHAGE IN RELATION TO CHEDDAR CHEESEMAKING¹

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Abstract

The sudden stoppage of acid development in several experimental vats of Cheddar cheese was shown to be due to the activity of a polyvalent streptococcal phage. Although the starter used consisted of a mixture of organisms, the stoppage was as abrupt as that in cases in which single strain starters are employed. Experimental infection of a vat of milk duplicated the original findings and resulted in the production of a cheese of poor body and texture, with a decidedly "fruity" flavour.

The presence of phage in the starter itself could not be demonstrated. Tests made on the milk of 18 cows, composite herd samples, stable and laboratory air, and rennet extract failed to indicate specifically the origin of the lytic agent.

Introduction

In the manufacture of Cheddar cheese a uniform development of lactic acid throughout the process is highly desirable. To ensure this, a quantity of "starter" consisting of a clotted milk culture of lactic acid streptococci is added to the milk. Failure of the starter to develop acid at the required rate disrupts the normal manufacturing process, causes considerable inconvenience, and lowers the quality of the cheese.

In New Zealand, failure to produce acid has been sufficiently common to justify extensive research directed to the elimination of this trouble. Whitehead and Cox (11, 12) have shown that such failures were frequently due to the sudden development of streptococcal bacteriophages that lysed the starter organisms and prevented the normal fermentation of lactose. Since the starters used in New Zealand are mainly prepared from single strains of *Streptococcus cremoris* (13), the appearance of a phage specific for the particular strain of streptococcus present in the starter results in complete lysis of this organism, either in the starter itself or at some stage in the manufacturing process. In view of the strain-specific nature of these streptococcal phages, the presence of phage rarely, if ever, results in as complete a failure of acid production with a mixed culture as it does with a single-strain starter (15).

Streptococcal phages are a common phenomenon in New Zealand (13), and they have also been demonstrated elsewhere. Mazé (5) has reported the presence of phages attacking the "normal lactic acid bacteria" in cheese factories in France. Davis (1) has recently reported the first case of phage activity in England, where single-strain cultures were being used. Nelson *et al.* (7) in Iowa have shown that slow acid production in butter cultures may be due to phage activity, but we have failed to find any reference to phage in

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connection with cheesemaking in North America. However, in the opinion of an outstanding authority (8), troubles attributable to phage may be much more general than is suspected. In view of the possible economic importance of the problem, the following account of an outbreak of phage infection at the Central Experimental Farm, Ottawa, may be of interest.

Description of the Outbreak

Acid development ceased abruptly in the early stages of manufacture in three vats of experimental cheese; in the fourth vat it slowed up later, the "milling" process being three hours behind the normal. Microscopic examination of the whey from these vats, after the stoppage occurred, revealed the complete disappearance of the starter organisms.

The starter employed, No. 21, consists of a mixture of various types of streptococci that may be distinguished from one another by length of chain formation, rate of acid production, and other physiological characteristics (Table I). It had been collected from a factory in Eastern Ontario in October, 1939 (2). Because of its strong acid production at 37° C., this starter was selected for distribution to cheesemakers throughout Eastern Ontario. During the five months up to the end of October, 1940, more than 250 cultures prepared from this starter had been supplied to 162 factories. This starter has also been employed continuously at the Central Experimental Farm Dairy for the ripening of cream for buttermaking, and in the manufacture of more than 60 vats of experimental cheese. At no other time had any weakening in its fermentative power been observed, nor has there been any word of this from the numerous factories using it.

Evidence of Phage Activity

That the abrupt cessation of acid production in the experimental vats was due to phage activity was first indicated by the disappearance of the starter organisms from the whey. Further confirmation was obtained in several ways. (1) Whey was passed through a sterile Seitz filter; a drop of diluted filtrate, when added to sterile milk freshly seeded with starter No. 21, caused virtually complete disappearance of the starter organisms within four and one-half hours' incubation at 32° C., whereas the control tube showed millions of streptococci per millilitre (Figs. 1, 2, and 3). The lytic agent could be transmitted indefinitely by inoculation into fresh milk seeded with this starter. (2) Plaque formation on yeast-extract-whey agar (Fig. 4) was demonstrated by means of the technique described by Whitehead and Cox (12). (3) Lysis in broth was demonstrated by the addition of aliquots of the whey filtrate to yeast-extract-whey broth cultures of 10 organisms isolated from this starter. As will be seen from Table I, 7 of the 10 cultures were completely lysed within six hours, while the remaining three showed partial lysis. (4) The substitution of a new starter (No. 2) for No. 21 in the experimental cheesemaking immediately following the phage attack resulted

TABLE I
SENSITIVITY TO LYSIS, MORPHOLOGY, AND PHYSIOLOGICAL CHARACTERISTICS OF STRAINS OF STREPTOCOCCI ISOLATED FROM STARTER No. 21

Strain No.	Degree of lysis*							Morphology**	Physiological characteristics						NH ₃ produced from peptone
	Incubation, hr.								Growth in		Growth at				
	2	3	4	5	6	7	Litmus milk		4% NaCl broth	pH 9.1	10° C.	40° C.			
													Reduction	Coagulation	
1	-	+++	++++	++++	++++	++++	Long and short chains	+	+	+	+	+	+		
6	-	-	++++	++++	++++	++++	Very long chains	-	+	+	-	-	+		
7	-	-	-	+	+	+	Pairs and short chains	Slight	+	+	+	+	+		
8	-	+++	++++	++++	++++	++++	Pairs and short chains, some long chains	+	+	+	+	+	-		
10	-	+++	++++	++++	++++	++++	Pairs, no chains	+	+	-	+	+	+		
12	-	-	+	+	+	+	Long chains, a few short chains	+	+	-	+	+	-		
15	-	+++	++++	++++	++++	++++	Long chains	+	+	-	+	+	+		
16	-	-	+	+	+	+	Long and short chains	+	+	-	+	+	+		
21-1	-	-	++++	++++	++++	++++	Short to fairly long chains	+	+	-	-	+	+		
21-2	-	-	+	+	+	+	Pairs, a few short chains	+	+	+	+	+	+		
Controls	-	-	-	-	-	-									
<i>S. lactis</i>							Pairs and short chains	+	+	+	+	+	+		
<i>S. cremoris</i>							Long chains	+	-	-	-	-	-		

** Milk cultures after 16 hr. at 22° C.

* Degree of lysis: + + + + = complete.

+ + + = almost complete.

+ + = decidedly clearer than control.

+ = slightly clearer than control.

- = same as control.

in perfectly normal acid development in the next 10 vats. (5) The ability of an active phage filtrate to stop acid production in the cheese vat was demonstrated experimentally. A small vat containing 400 lb. of milk was inoculated with 4 lb. (1%) of a clotted milk culture of starter No. 21. After thorough mixing, one-half of the milk was transferred to an identical vat. Both vats were handled as uniformly as possible except that the wooden rake used to agitate the milk and curd in one vat, *F*, was lightly swabbed with a little of the phage-containing filtrate. The control vat (*C*) behaved normally throughout and produced a first grade cheese. On the other hand, in the phage-contaminated vat, acid development ceased immediately after cutting. The first titration of the whey showed 0.13% acidity; four hours later the acidity was unchanged. Microscopic preparations and Burri slants prepared from both vats shortly after the stoppage was noted showed millions of starter organisms in the whey of the control vat, none at all in that of vat *F*. Although some slight acid development, due to the action of non-starter organisms, was noted many hours later in vat *F*, the cheese was practically ruined.

After holding for 18 days at 15° C., the two vats were scored as follows*:

Vat	Flavour	Texture	Closeness	Colour	Finish	Total score
Control (<i>C</i>)	39.5	24.2	15.0	10.0	5.0	93.7
Filtrate (<i>F</i>)	37.0	23.0	14.5	9.5	5.0	89.0

* The Dominion Grade Standards specify the following minimal scores for cheese:

	Flavour	Total score
First Grade	39	92
Second Grade	37	87
Third Grade	<37	<87

The pronounced "fruity" flavour that lowered the score of cheese from vat *F*, appeared to be due to the large number of yeasts in the cheese (1,600,000 yeasts per gm.), whereas that from the control vat (*C*) made from identical milk contained 11,000 per gm. Plates poured on tryptone-glucose-skim-milk agar containing brom-cresol purple indicator showed a typical lactic acid flora in the control cheese (vat *C*) but almost complete absence of these organisms in the phage contaminated cheese (Figs. 5 and 6).

Further Studies with Phage

It has been previously mentioned that the bacteria-free filtrate of the original whey was active against all 10 strains of organisms isolated from starter No. 21. To determine whether the cause of the trouble was an unusually polyvalent phage or a mixture of races, a lysed yeast-extract-whey broth culture of Strain 8 was filtered through a sterilized Seitz filter, and the filtrate added to a freshly seeded broth culture of the same organism. This process was

TABLE II

EFFECT OF ADDING ACTIVE FILTRATE FROM STRAIN 8 TO 11 STOCK STARTERS, AS SHOWN
BY MICROSCOPIC EXAMINATION OF MILK
CULTURES AFTER SIX HOURS AT 32° C.

Starter No.	Control	Filtrate added	Morphology
1	++++*	++++	Pairs and short chains
8	++++	++++	Long chains
20	++++	++++	Pairs and short chains
21	++++	—	Long chains
30	++++	++	Pairs and short chains
31	++++	++++	Pairs and short chains
32	++++	++++	Pairs and long chains
33	++++	++	Pairs and short chains
34	++++	++++	Pairs and short chains
35	++++	++++	Pairs and long chains
36	++++	+++	Pairs and short chains

* Plus signs indicate approximate abundance of organisms observed.

repeated three times and the filtrate added to sterile milk seeded with starter No. 21. After eight hours' incubation, the organisms had completely disappeared. Similar results were obtained after 12 additional serial passages of the active filtrate at the expense of Strain 8. This would indicate that the active agent was a polyvalent phage rather than a mixture of races, as it would be expected that 15 serial transfers would have diluted out any races not specific for Strain 8.

To obtain further proof of the polyvalency of this lytic agent, the filtrate from the 15th transfer was tested against broth cultures of the original 10 strains (Table I). Seven of these (No. 6, 7, 8, 10, 15, 16 and 21-1) showed evidence of lysis. These represent organisms of varying morphology and physiological reactions (Table I). This reinforces the evidence mentioned in the previous paragraph suggesting that the phage in question is polyvalent.

To discover whether the phage would affect streptococci in other mixed culture starters the sterile filtrate from a lysed culture of Strain 8 was tested against milk cultures of 11 starters being carried in these laboratories. The results are presented in Table II. It will be observed that in three of these cultures there was evidence of inhibition of growth. Although a milk culture of starter No. 21 is characterized by the presence of very long chains of streptococci, the activity of this phage was not confined to strains with this characteristic. Starters No. 30, 33, and 36 contained predominantly short-chain streptococci, yet all three showed reduction in numbers of organisms, whereas No. 8 and 35, predominantly long-chain types, showed no such decrease. This is in accord with the results reported in Table I, where strains of both long and short-chain streptococci were completely lysed.

The potency of the phage present in a lysed culture of Strain 8 was determined, using decimal dilutions in yeast-extract-whey broth seeded with a broth culture of the susceptible organism. The following results were obtained:

Incubation period, hr.	Concentration of filtrate							
	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
	Degree of lysis							
6	++++	++++	++++	++++	+++	+++	+	-
18	++++	++++	++++	++++	+++	++	+	-
24	++++	++++	++++	++++	+++	++	++	-

In the cheese factory it is customary to use the same container for measuring out both cheese colour and rennet. After adding the colour to the milk just before "setting" the vat with rennet, the container is customarily rinsed in the milk, then in water. If phage is present in the milk, the container is quite likely to become contaminated. Rennet extract is next poured into the container, and, if an excess is introduced, the surplus is poured back into the vessel holding the rennet supply. In this way the rennet itself may become contaminated with phage. Unfortunately, the rennet extract was not tested for the presence of phage until 10 days after the outbreak; negative findings at that time do not preclude the possibility that the phage had died out in the interim.

The survival of phage in the cheese itself was investigated. Decimal dilutions of the cheese from vat *F*, after 18 days' ripening at 15° C., were added to sterile milk tubes seeded with starter No. 21; subcultures were later made from these. No evidence of lysis was obtained even in the lowest dilution (10⁻¹); this suggested that the phage had died out during this period.

Source of the Phage

The actual source of these streptococcal phages is still a matter of opinion. Whitehead and his co-workers (12, 13, 14) concluded that the streptococcal phages originate within the single strain starters themselves. External contamination was ruled out mainly on the grounds of the relatively high degree of specificity exhibited by the races of phage they studied. In a more recent publication (16), however, reference is made to the possible contamination of starter cultures with phage within the factory, since cheese whey commonly contains a high concentration of phage (14). Mazé, on the other hand, while confirming the specificity of the races of phage, believes the trouble arises from external contamination within the cheese factory (6). While admitting that the origin of the phage is still obscure, he suspects the raw milk because of its frequent contamination with organisms from manure (5). Since starter organisms (*Streptococcus lactis* and *S. cremoris*) are not found in bovine faeces (9), it seems unlikely that this would be an important source of the phage specific for these organisms.

Starter

In view of the emphasis placed by the New Zealand workers upon the starter itself as the source of phage, a number of attempts were made to

demonstrate its presence. There is evidence that some milks favour the development of phage whereas others appear to inhibit it (12, 13). It has also been shown that conditions (use of small inocula, aeration of milk) that prolong the lag phase of growth of the starter organisms, facilitate the demonstration of phage that may be present in the starter. Consequently, 125 ml. portions of milk were obtained from each of 12 cows in various stages of lactation, and with udders of varying degrees of abnormality. These milks were autoclaved at 15 lb. pressure for 20 min.; after cooling to 30° C., they were lightly seeded with a six-day-old starter culture and aerated aseptically by passing a stream of air through the flasks for 15 hr. Microscopic preparations showed abundant growth in every case. This was also true for tubes of sterile milk inoculated from the 15 hr. flask cultures. These all clotted normally, as did a second set of milk tubes inoculated from the first.

Tests were also made with a number of autoclaved or pasteurized samples representative of the milk of the entire herd. In only one instance was there any indication of the presence of phage. In the one exception, 400 ml. of milk pasteurized at 62° C. for 30 min. was seeded with a five-day-old starter culture. After incubation for one hour, calcium chloride solution and rennet extract were added to coagulate the milk. The whey that extruded on standing at 37° for 30 min. was filtered through paper, then through a sterilized Seitz filter. Three-millilitre portions of whey filtrate were added to yeast-extract-whey broth cultures of the 10 organisms reported in Table I. After six hours' incubation, Strains 8 and 15 showed lysis.

This finding cannot, however, be regarded as proof of the presence of phage in the starter itself. In the first place, the heat treatment employed in pasteurization would not destroy phage present in the raw milk (13). Secondly, the rennet had been exposed to phage contamination in the manner described in a previous section. Thirdly, there exists the possibility of accidental contamination. A repeat test was made a few days later using milk steamed for 45 min. and aerated aseptically during incubation for six hours with the starter inoculum. No lysis was noted when the whey filtrate was tested against a broth culture of Strain 8, nor when this culture was filtered and added to a fresh culture. As previously mentioned, phage could not be demonstrated in the rennet extract.

Negative results were also obtained with several samples of milk from the factory supplying the milk in which the phage activity was first demonstrated. The vitality test of Whitehead and Cox (10) was conducted concurrently on portions of milk from both normal and abnormal vats on the day that trouble was experienced. Although the same lot of starter was employed as in the vats in which phage developed, there was no evidence of phage action in the vitality test.

That the milk supply ordinarily used in preparing the starter was suitable for the demonstration of phage active against starter No. 21 has been indicated earlier. Repetition of the first experiment by adding low dilutions of active

filtrate to sterile milk inoculated with starter cultures from three to six days old gave identical results, i.e., complete disappearance of starter organisms.

Milk Supply

While it seems unlikely that milk from individual cows would contain phage active against starter No. 21, tests were conducted upon samples obtained from 18 cows in the Central Experimental Farm herd. Samples obtained from the milkers' pails were seeded lightly with an old culture of starter and aerated aseptically overnight. Next morning, transfers were made from the original samples to sterile milk tubes freshly seeded with the starter; a second transfer into similar milk tubes was made from the first tubes after eight hours' incubation at 32° C. Although none of the original samples showed any evidence of phage activity, the tube transfers from two of these milks did show lysis. Seitz filtrates obtained from these were added to sterile milk seeded with starter and lysis was again noted. To confirm the presence of phage in the milk of these two cows, further samplings of aseptically drawn fore-milk and of milk from the pail were tested at 20 subsequent milkings. In no instance was there any evidence of phage.

Air

Tests for the presence of phage in the stable air were made upon two occasions by exposing a 15 sq. in. surface of sterile milk for 10 to 20 min. during milking. The results were negative. A similar exposure test in the laboratory (four weeks after the outbreak) yielded negative results.

Discussion

The outbreak of phage infection described is of particular interest in that the stoppage of acid fermentation in vats seeded with a mixed starter was as abrupt and complete as any reported for single strain starters. As Whitehead and Hunter (15) have remarked, this rarely, if ever, happens. In New Zealand, cheese milk is flash-pasteurized to destroy the bulk of contaminating bacteria before the starter is added. If the phage attacking the single strain culture of *Streptococcus cremoris* is present in amounts sufficient to lyse these organisms completely, subsequent acid production would necessarily be very slow. Although the milk in our experimental vats was not pasteurized, the bacterial content was fairly low, only one of the four vats reducing resazurin to the pink stage (3) within five hours. Consequently, when the various strains of streptococci in the starter were lysed, there was no further acid development until the contaminating organisms present in the original milk (mostly micrococci) reached sufficient numbers.

In view of the New Zealand findings, it is also noteworthy that we have failed to demonstrate with certainty the presence of phage in the starter itself, when grown in a variety of milk supplies, and under conditions conducive to its appearance. However, in view of Whitehead and Hunter's (13) observations concerning the "periodic sensitivity" of starters for phage demonstration, it

may not be safe to conclude that the starter in question is at all times completely free from phage.

The writers' inability to demonstrate phage in so many subsequent samplings of milk from the two cows in whose milk phage was detected on a single occasion leads to the suspicion that the positive findings were due to accidental contamination during the inoculation and subsequent transfers of these two samples. The ease with which phage contamination may occur in a laboratory is well known (4). In a recent report (16) Whitehead and Hunter remark, "A few further experiments served to show quite clearly that, in the commercial factories, contamination of the cultures with phage occurred through the atmosphere even when a normal bacteriological technique was practised It is evident now that the technique normally considered adequate to prevent infection of cultures is not efficient under certain circumstances in preventing the access of bacteriophage to cultures."

The original source of the phage in the present outbreak thus remains obscure. Milks from a variety of sources had been employed in experimental cheesemaking just prior to the outbreak. Any one of these supplies may have introduced the phage, which would then have been carried over from day to day on the wooden rakes and other equipment. Since it could not be ascertained which herds furnished these supplies further investigations in this direction could not be pursued.

Slow acid development is not uncommon in Eastern Canada although no previous investigations regarding phage activity have been reported from this area. Cheesemakers admit having experienced trouble which they have attributed to some inexplicable failure of the starter. The substitution of a different starter generally corrected the trouble and the cheesemaker's diagnosis appeared to have been fully substantiated. That the fault may not be in the starter itself, however, is suggested by the present experience. If the maker experiencing trouble due to phage activity happens to replace his starter with a culture of similar bacterial composition, he will almost inevitably experience further trouble and unjustly blame the new starter. This might easily happen with a maker using No. 21, which is still being distributed regularly. That the trouble may not be inherent in the starter itself should be impressed upon all cheesemakers, lest the possible consequences prove embarrassing to those responsible for the distribution of starters.

While such an abrupt and complete stoppage of acid development as encountered by the writers may not be common in commercial factories, slow acid development, frequently resulting in "fruity" flavour, is encountered occasionally. The writers' experiences, in which three out of five phage-contaminated vats were graded down for "fruity" flavour, suggest that phage contamination may be concerned in the slow acid development. Where this occurs, it would be wise to change to a different starter, and to subject the premises and equipment to a very thorough cleaning, followed by sterilizing treatment with a hypochlorite solution. This procedure has been reported as effective in

eliminating the trouble in several instances (8). Further studies dealing with this phase of the subject are projected.

That air-borne contamination with phage is a significant factor is indicated in a report from New Zealand just published (17). By the provision of a special starter-room separate from the factory, failures of starters due to phage contamination were completely eliminated at this particular factory throughout the season. The centrifugal separator used in removing the butter-fat from the whey was found to emit finely atomized whey and this proved to be the major source of the air-borne phage in the factory.

Acknowledgments

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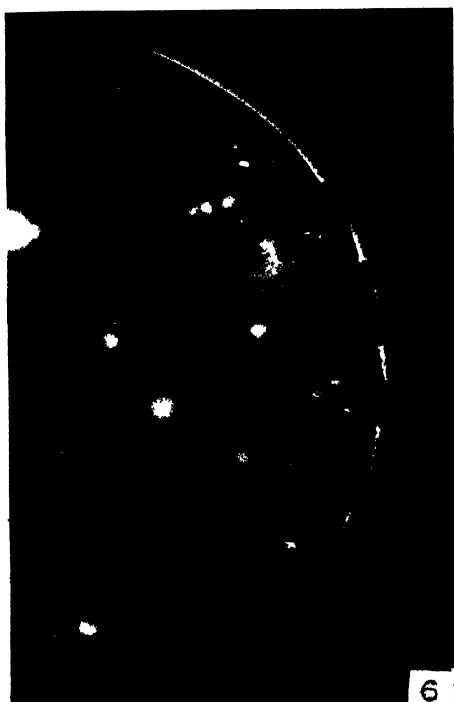
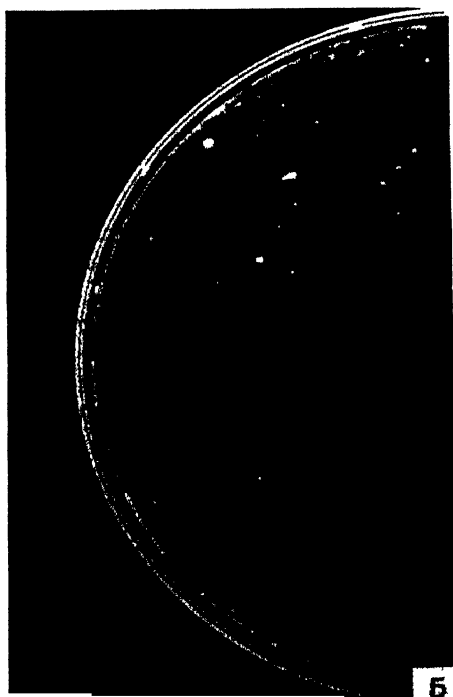
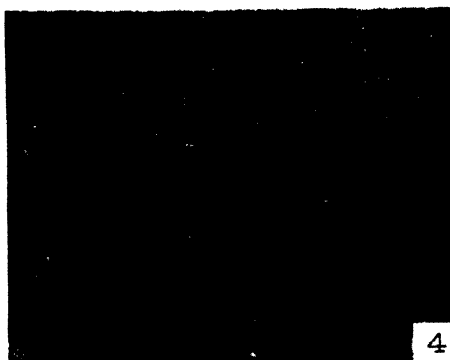
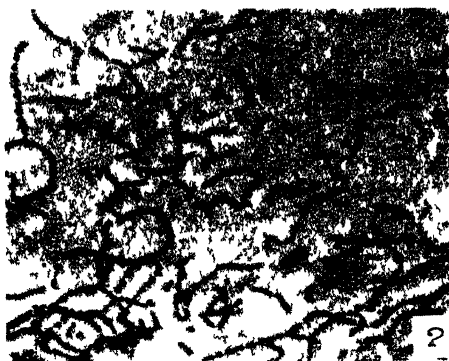
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EXPLANATION OF PLATE I

FIG. 1. Sterile milk inoculated with starter No. 21; before incubation. FIG. 2. Same milk after four and one-half hours' incubation at 32° C. FIG. 3. Similarly inoculated milk plus phage after four and one-half hours' incubation at 32° C.

(FIGS. 1-3. Photomicrographs of microscopic preparations, Breed-Newman stain. $\times 1000$.)

FIG. 4. Portion of plate culture of starter No. 21 seeded with active filtrate, showing phage plaques. Natural size. FIG. 5. Plate poured from 1×10^{-7} dilution of cheese from control vat. Tryptone-glucose-skim-milk agar; incubation period, five days at 30° C. Natural size. FIG. 6. Plate poured from 1×10^{-7} dilution of cheese from phage contaminated vat. Tryptone-glucose-skim-milk agar; incubation period five days at 30° C. Natural size.



ÉTUDES SUR LES HYBRIDES DE CISTES VI. INTROGRESSION DANS LA SECTION *LADANIUM*¹

PAR PIERRE DANSEREAU²

Sommaire

Certaines méthodes appliquées à la taxonomie permettent d'apprécier le mode de la genèse des divers groupes. Des études précédentes ont défini le mécanisme de l'introggression: persistance après hybridation de certains caractères d'une espèce dans une autre. Ces notions appliquées à la section *Ladanium* du genre *Cistus* indiquent que le *C. ladaniferus* var. *petiolatus*, localisé en Afrique du Nord, a eu comme origine une contamination du *C. ladaniferus* par le *C. laurifolius*.

Identification des mécanismes de l'évolution

Le processus de la différenciation, qui a pour résultat l'élimination progressive des virtualités et la spécialisation de plus en plus étroite des formes vivantes, s'accomplit par des mécanismes divers agissant séparément ou ensemble et éprouvant de la part du milieu plus ou moins de résistance. La diversité des modalités mises en cause et de leur conjonction engendre des séries de variations non équivalentes sur lesquelles sont basées les entités taxonomiques.

La matière végétale a apparemment produit des formes nouvelles par quatre mécanismes principaux: mutation, polyplôidie, hybridation, sélection.* Les données que nous possédons actuellement sur la fréquence des mutations dans la nature sont sans doute insuffisantes pour permettre des généralisations, surtout en l'absence de la connaissance des causes naturelles susceptibles de les provoquer. Sugiura (14) a cependant trouvé que, sur 80 familles, représentées par 250 espèces, celles dont le nombre respectif est de n et $n + 1$ ou $n - 1$ manifestaient entre elles plus d'affinité que celles à n et un multiple de n . Les mutants aneuploïdes s'écarteraient donc moins du phylum primitif que les polyplôïdes.

En fait, les mutations simples (sans polyplôidie ou aneuploïdie) sont accompagnées, la plupart du temps, de variations morphologiques peu importantes, portant sur des caractères qui engendrent des formes et des variétés seulement.

Tandis que, dans certains groupes le doublement du nombre chromosomique entraîne la ségrégation générique, spécifique, ou variétale, tandis que dans

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* C'est là une classification surtout pratique puisque la mutation peut être génique ou chromosomique, (et peut donc inclure la polyplôidie, et que la sélection est une conséquence ordinaire de l'hybridation.

d'autres groupes elle ne s'accompagne apparemment d'aucune variation morphologique (10).

L'hybridation se produit à tous les degrés de l'échelle taxonomique. On lui a donné le nom de métissage quand elle se manifeste à l'intérieur d'une espèce. Cette distinction ne vaut évidemment, à un point de vue strictement objectif, que ce que vaut l'espèce linnéenne elle-même. On constate que certaines variétés d'une même espèce ne se croisent pas; tandis que les variétés d'une autre espèce sont rendues à ce point instables par la fréquence de leurs croisements qu'on peut douter de leur existence et considérer l'espèce globale comme un tout délimité quant à l'extérieur, mais où la différenciation est trop peu avancée à l'intérieur pour l'avoir compartimentée (*Cistus salviifolius*) (8).

Il faut donc, pour qu'une entité taxonomique soit légitime, qu'elle ait une base naturelle reconnaissable aux barrières qui l'isolent morphologiquement et génétiquement des entités affines. Un croisement est théoriquement suivi d'un grand nombre de recombinaisons, associant les divers caractères des deux groupes originaux en des assemblages nouveaux. Il a été prouvé, dans la pratique, qu'un très petit nombre de ces possibilités se réalise, pour des raisons génétiques. Anderson a même formulé une loi de la recombinaison (2).

Il n'est pas moins clair que le milieu impose à son tour des limitations très étroites. La sélection opère tout de suite, et il ne semble pas douteux que, parmi les populations naturelles, le retour aux formes parentales originelles soit la règle. Ce retour est, dans certains cas, très rapide (1). Certains croisements entre espèces sont génétiquement aussi faciles et aussi fertiles que les fécondations légitimes (surtout dans le cas de plantes autostériles (7)). Dans le cas des *Cistus monspeliensis* et *salviifolius*, par exemple, le croisement est très fréquent. Certes, l'hybride auquel ces deux espèces donnent naissance (\times *C. florentinus*) n'est pas rare dans la nature, sauf en Afrique du Nord, mais il n'atteint pas une importance numérique notable, sauf dans des conditions spéciales sur lesquelles nous aurons l'occasion de revenir*. Les recombinaisons sont nettement différentes selon les lieux (8). La sélection agit donc localement sur les populations végétales et élimine certains caractères en présence, soit qu'elle agisse sur une espèce pure dont elle utilise ce qui lui reste d'hétérozygotie, soit qu'elle choisisse dans le stock plus riche d'un hybride, toujours avec la tendance vers l'équilibre homozygote et la stabilité cyto-nucléaire. Ce travail peut être contrarié par la formation de nouveaux individus hybrides ayant la même formule et tendant à ramener toujours l'évolution que dirige la sélection à son point de départ, ou favorisé par la disparition accidentelle de certaine catégorie d'individus, par exemple de l'un ou des deux parents, s'il s'agit d'un hybride, ou de l'espèce-type s'il s'agit d'une espèce pure. C'est vraisemblablement le cas des *Pteridium latiusculum* et *aquilinum*, du *Pinus laricio* et de ses variétés où la barrière géographique a aidé le travail de la sélection et de la conservation locale des mutations (4).

* Voir: *Études sur les hybrides de Cistes. V. Comportement du \times C. florentinus Lam. (en cours de préparation).*

Il n'est pas exagéré de prétendre que nous connaissons les voies de l'évolution et qu'il nous est possible de déceler le mécanisme ou les mécanismes formateurs d'entités nouvelles. Il est intéressant de noter, ici comme ailleurs, que le travail accompli est sans relation nécessaire avec l'importance du résultat et il semble qu'il ne faut pas plus de travail pour produire une forme qu'une espèce. Quant à l'action de la sélection naturelle, elle est à la fois plus aléatoire et plus difficile à observer, puisque, dans presque tous les cas, le facteur "temps" dépasse de beaucoup l'échelle de nos observations.

La sélection agissant au sein de groupes complexes comme les *Rosa*, *Rubus*, *Mentha*, *Euphrasia*, n'est guère décelable. Par contre, elle devient sensible dans les groupes moins riches en espèces, moins polymorphes ou, en tous cas, dans des groupes où sont en jeu un certain nombre de caractères qualitatifs, et en présence de zones de discontinuité strictement observables.

Les auteurs ont émis des opinions diverses sur l'importance de l'hybridation dans l'évolution. Lotsy, par exemple, lui assigne le rôle principal (11). Mais, comme le faisait remarquer Anderson (1), cette importance ne sera mesurable que lorsque nous aurons acquis une connaissance concrète des effets de l'hybridation dans la nature. Il importe donc surtout, pour l'instant, d'accumuler des documents sur cette question, en vérifiant la constance du mécanisme dans divers groupes taxonomiques. La statistique des populations naturelles et l'étude cytologique des hybrides sont appelés à nous fournir les plus précieux indices sur les relations de l'hybridation et de la sélection.

La signification de l'introgression

Un aspect particulier de la sélection naturelle est le mécanisme de l'introgression, défini explicitement par Anderson dans une étude sur les hybrides de *Tradescantia* (1). Ce phénomène a été constaté depuis chez d'autres genres: *Aster* (15), *Iris* (12), et *Cistus* (5). L'introgression est le passage d'un ou de plusieurs caractères d'une espèce dans une autre à la suite d'hybridations. C'est un mécanisme qui favorise la différenciation d'un certain nombre d'individus d'une espèce dans un sens qui les rapproche d'une autre espèce existante. Il induit donc la convergence et se présente dans la phylogénie comme une anastomose.

Certes, les cas d'évolution parallèle ne sont pas rares et chaque fois qu'on trouve un caractère d'un groupe dans un autre groupe, il est imprudent de conclure à l'hybridation. Ne voit-on pas des *Populus* européens et américains produire des formes analogues à partir eux-mêmes de types peu divergents? De même les formes épineuses et inermes chez les *Rosa* de l'Amérique du Nord résultent aussi vraisemblablement des gènes latents dans le genre entier ou de mutations habituelles (voir aussi le feuillage panaché chez l'*Acer negundo*) que de l'hybridation. Pour d'autres cas, le rôle de l'hybridation est évident, comme pour l'évolution parallèle des *Circaea* européens et américains.

Anderson a proposé un système qui permet de mesurer la gravité de l'influence exercée par l'hybridation, en convertissant les données qualitatives

sous une forme quantitative (1). Il a fourni lui-même une première application de son principe en analysant des populations naturelles du *Tradescantia canaliculata*, du *T. virginiana* et de leurs hybrides.

L'expérience d'Anderson a été répétée par Riley sur des *Iris* (12) et des *Tradescantia* (13) de la Louisiane, par Wetmore et Delisle sur des *Aster* (15), par Goodwin sur des *Solidago* (10), et par l'auteur sur des *Cistus* (5).

Les résultats obtenus jusqu'ici permettent de donner à une espèce une expression mathématique et graphique, au moins en ce qui concerne sa position relativement à une autre espèce. Ils permettent, en outre, d'apprécier le degré d'éloignement des individus hybrides par rapport à chacun des parents, et de constater lequel des deux est favorisé dans la recombinaison. Dans la plupart des cas, l'un est à ce point favorisé que ce qui reste de l'autre après la recombinaison est trop insignifiant pour mériter un rang taxonomique (3).

Aucun des auteurs précités n'a voulu accorder un status précis aux spécimens non-typiques ou non franchement intermédiaires. En ce qui concerne le *Cistus monspeliensis* l'auteur, n'a pas voulu non plus accorder de valeur taxonomique à l'introggression de quelques petits caractères du *C. salvifolius* (5).

Il semble probable qu'il faut, pour qu'un caractère introgressif se fixe, la collaboration d'autres causes, la disparition accidentelle de populations pures susceptibles de *noyer* ce caractère ou la répétition, pendant une période très longue, du même phénomène avec élimination graduelle des autres types. Je crois que c'est ce dernier cas qui se présente dans la section *Ladanium* du genre *Cistus*.

Dynamisme de la section *Ladanium* du genre *Cistus*

Cette section se compose de deux espèces, les *C. laurifolius* et *ladaniferus* (6).

Le *C. laurifolius* L. a des feuilles longuement pétiolées, le plus souvent cordées, très larges (18 à 30 mm.), à marge le plus souvent ondulée. Son inflorescence porte cinq à huit fleurs en ombelle, à pétales blancs de 20 à 30 mm. de longueur; la pubescence du calice se compose de poils simples assez longs; les loges de l'ovaire sont au nombre absolument invariable de cinq. Il se trouve en des stations isolées en Asie Mineure, en Italie, en France méridionale, et assez abondamment en Espagne et au Portugal (Fig. 3).

Le *C. laurifolius* L. var. *atlanticus* Pit. (Atlas et Corse) (Fig. 3) diffère du type par des caractères exclusivement quantitatifs, i.e., feuilles et sépales plus petits, etc.

Le *C. ladaniferus* L. typique (var. *albiflorus* Dun. emend P. Dans.) a une inflorescence solitaire, faible et plus ou moins retombante, couverte de larges bractées; les sépales ne portent pas de poils simples, mais des poils étoilés d'une forme très spéciale dite "en écusson"; les loges de l'ovaire sont au nombre de 10; les pétales sont très grands (30 à 35 mm.) et portent parfois une macule pourpre à la base. Les feuilles sont sessiles, linéaires ou lancéolées, larges de

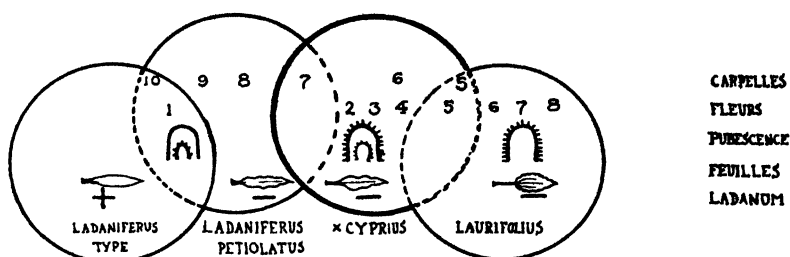


FIG. 1. Répartition des caractères dans la section *Ladanum* du genre *Cistus*. Nombre de carpelles (de 5 à 10); nombre de fleurs (de 1 à 8); pubescence simple (à droite, chez le *C. laurifolius*), en "écusson" (à gauche, chez le *C. ladaniferus*) ou des deux types (X *C. cyprius*); feuilles sessiles (*C. ladaniferus* type) à longuement pétiolées (*C. laurifolius*); feuilles planes (*C. ladaniferus* type) à fortement ondulées (*C. laurifolius*); présence (+) ou absence (-) de ladanum.

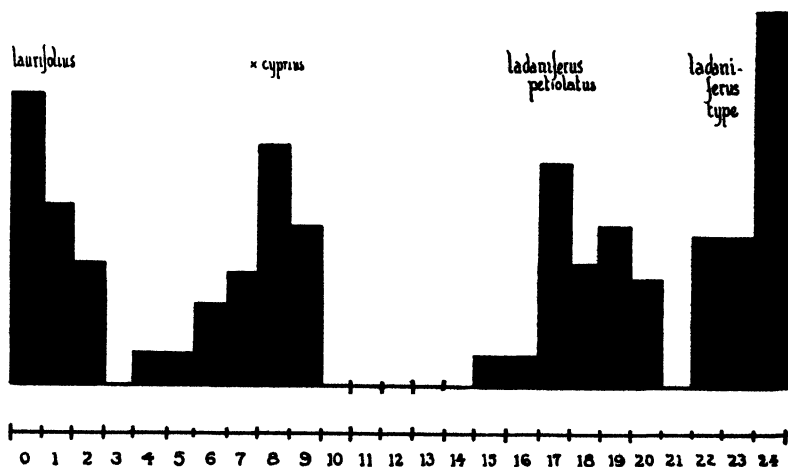


FIG. 2. Profil statistique des quatre groupes constituant la section *Ladanum*. Des caractères qualitatifs ont reçu une valeur quantitative permettant d'établir les positions relatives des quatre groupes taxonomiques.



FIG. 3. L'aire de distribution de la section *Ladanum*.

10 à 21 mm. à marge plutôt plane, enduites d'une résine appelée "ladanum". Il est très répandu en Espagne et au Portugal et on le trouve localement en France méditerranéenne.

Les *C. ladaniferus* de l'Afrique du Nord appartiennent tous à la variété *petiolatus* Maire, qui diffère du type par les caractères suivants: loges à l'ovaire 7 à 10, feuilles courtement pétiolées, plutôt ondulées, sans ladanum.

Le \times *C. cyprius* Lam. (*C. ladaniferus* \times *C. laurifolius*), d'autre part, peut se reconnaître à ses 5 à 7 loges à l'ovaire, ses sépales portant les deux types de pubescence mentionnés plus haut, à ses fleurs dont le nombre varie de deux à cinq, à ses feuilles pétiolées assez longuement et de contour plus ou moins ovale, ne sécrétant pas de ladanum. On le rencontre fréquemment dans les jardins et on l'a souvent trouvé à l'état sauvage, en Europe et en Afrique, dans la zone habitée par les espèces parentes.

La Fig. 1 fait voir les associations de caractères telles qu'on les trouve réalisées dans la nature. Les combinaisons de facteurs sont si loin d'être libres que les quatre entités principales de la section *Ladanium* manifestent une parfaite discontinuité et se révèlent extrêmement faciles à caractériser. Les frontières indiquées pour chaque groupe sont absolues et basées sur l'observation d'un grand nombre de spécimens.

La Fig 2 est une transposition de la Fig. 1, basée sur la méthode statistique citée plus haut. Les pôles sont, d'une part le *C. laurifolius* et d'autre part le *C. ladaniferus* typique. Sept séries de caractères ont été considérées; une valeur relative a été donnée à chaque modalité de chaque série, élevée si elle faisait partie du complexe "*ladaniferus*", nulle si elle faisait partie du complexe "*laurifolius*" et plus ou moins élevée selon qu'elle se rapprochait ou s'éloignait du pôle "*ladaniferus*". Le tableau I énumère ces caractères et donne la valeur qui leur a été assignée dans la Fig. 2.

La valeur idéale du *C. laurifolius* sera donc de 0, et celle du *C. ladaniferus* typique de 24, ceci étant donné que les caractères mentionnés sont absolus pour chaque espèce. Les caractères de feuilles, cependant (séries V et VI) sont un peu moins absolus, et dans l'ensemble des spécimens que j'ai pu examiner c'est faire amplement la part des choses que d'admettre que 40% ont la marge ondulée; et que d'admettre, d'autre part, que 29% des *C. ladaniferus* européens ont une marge ondulée et 20% une feuille de 5 à 10 mm.

Etant donné la valeur constante des séries I, II, III, IV, et VII pour les *C. laurifolius* et *ladaniferus* var. *albiflorus*, on peut admettre les figures ci-dessus comme profil de ces groupes taxonomiques, au moins en ce qui concerne les caractères qui les séparent, sans recourir à un examen statistique qui ne saurait donner un résultat sensiblement différent de celui que je propose. La valeur de 0 pour les deux séries (V et VI) n'a jamais été observée chez un *C. ladaniferus* typique et même une valeur de 1 pour chaque série chez un grand nombre de *C. ladaniferus* var. *albiflorus* ne changerait pas sensiblement la position de ce groupe sur le graphique, puisqu'on n'enregistrerait en aucun cas de valeur inférieure à 22. De même pour le *C. laurifolius* qui ne s'écarte

de la valeur 0 que pour la série V, ce qui rend impossible pour lui toute valeur supérieure à 2. Les deux masses, donc, qui occupent la droite et la gauche de la Fig. 2 constituent chacune un ensemble cohérent et constant et expriment la réalité de tous les individus des groupes en question. (Voir description (6).)

TABLEAU I

Numéro de la série	Caractères	Valeur relative
I	Nombre des carpelles	
	10	4
	9	3
	8	2
	7	1
	6	1
	5	0
II	Nombre de fleurs	
	1	4
	2-4	2
	+4	0
III	Poils des sépales	
	en écusson	4
	des deux types	2
	simples	0
IV	Longueur des pétioles	
	nulle	4
	-5 mm.	2
	5-10 mm.	1
	10-25 mm.	0
V	Marge des feuilles	
	plane	2
	ondulée	1
	crépue	0
VI	Largueur des feuilles	
	1-5 mm.	2
	5-10 mm.	1
	10-20 mm.	0
VII	Présence de ladanum	
	présent	4
	absent	0

Quant aux deux autres groupes déjà représentés dans la Fig. 1, ils ont été l'objet, pour tous les caractères mentionnés, d'un relevé statistique. La position qui leur est assignée ici résulte d'observations faites sur 18 spécimens du $\times C. cyprius$ (*C. ladaniferus* \times *C. laurifolius*) et 54 spécimens du *C. ladaniferus* var. *petiolatus*, le tout étant rapporté à 100 sur le graphique.

La première conclusion qui se dégage de cette figure est la parfaite discontinuité qui existe entre les quatre entités. Quand on songe qu'il s'agit de sept séries de caractères, cette discontinuité est sans doute très remarquable

et en tout cas suffisante pour permettre d'individualiser les groupes, de leur conférer une identité.

La position du $\times C. cyprius$ —40% portant l'indice 8—est assez significative au point de vue génétique, puisqu'elle nous indique que, dans l'ensemble, les caractères du *C. laurifolius* sont dominants. On voit en effet sur la Fig. 1 que jamais le $\times C. cyprius$ n'a plus de sept loges à l'ovaire; qu'il n'a jamais moins de deux fleurs; que ses feuilles sont toujours pétiolées et toujours plus larges que de 5 mm. Le matériel examiné était nord-africain aussi bien qu'eupéen, et cependant les valeurs sont essentiellement les mêmes puisqu'aucun spécimen n'a révélé un indice entre 10 et 14. Le matériel africain a toutefois donné les plus hautes valeurs pour les séries I et II.

Quant au *C. ladaniferus* var. *petiolatus*, s'il n'a aucun point de contact avec le type, il est, d'autre part, totalement dégagé de la zone d'hybridation hors de laquelle aucune plante ne saurait être distinguée de l'un ou de l'autre parent. Si on ne connaissait la valeur dominante de certains facteurs du *C. laurifolius*, on pourrait théoriquement étendre cette zone jusqu'à l'indice 12; mais même alors, on n'atteindrait pas le niveau inférieur des *C. ladaniferus* de l'Afrique du Nord. La question ne se pose pas, d'ailleurs, si l'on peut considérer ce groupe comme hybride puisqu'il constitue de vastes populations en Afrique du Nord, en l'absence totale du type (*C. ladaniferus* var. *albiflorus*) et qu'il s'hybride à son tour, avec le *C. laurifolius* et donne naissance à des $\times C. cyprius$ ayant un indice de 8.

L'hypothèse donc qui se présente le plus naturellement est la suivante. En Afrique du Nord, le *C. ladaniferus* a été contaminé, à un moment de son histoire, par le *C. laurifolius*. Cette hybridation et les recombinaisons subséquentes ont engendré la formation d'un groupe nouveau, retenant quelques-uns des caractères du *C. laurifolius*, ou plus exactement des caractères intermédiaires entre les deux espèces. Ce groupe nous apparaît aujourd'hui complètement stabilisé, puisqu'il n'existe plus d'individus appartenant au *C. ladaniferus* typique, en Afrique du Nord. Il se rapproche du *C. laurifolius* sans que cet écart soit assez grave pour le faire sortir de l'orbite de l'espèce-mère (*C. ladaniferus*).

La section *Ladanium* est donc un complexe bipolaire où la différenciation s'est faite au moyen de l'introgression.

Ce phénomène est sans doute assez commun dans les genres où les espèces s'hybrident facilement. Il s'est produit chez les *Tradescantia*, les *Iris*, et les *Aster*, et chez les autres espèces de *Cistus*. Mais on n'a pas signalé jusqu'ici que ce mécanisme ait engendré une entité taxonomique nouvelle, du même ordre de grandeur et aussi indiscutable que celle-ci. Riley, par exemple, n'approfondit pas l'identité taxonomique des *Iris* ayant des indices intermédiaires entre les *I. fulva* et *I. hexagona* var. *giganticoerulea* (12). Il n'est surtout jusqu'ici question nulle part de variétés à la fois morphologiquement bien caractérisées et géographiquement isolées, s'étant stabilisées par la sélection naturelle.

Il deviendra sans doute intéressant d'appliquer la présente méthode à beaucoup de variétés et même d'espèces décrites et acceptées par les taxonomistes. L'auteur ne croit pas que l'origine hybride, même relativement récente, infirme la validité d'un groupe taxonomique. Il pense à des formes comme les *Populus alba* var. *denudata*, *Cornus canadensis* var. *intermedia* et à des entités analogues qui se rapprochent visiblement d'une espèce voisine, quoique on puisse les considérer comme fixées à l'heure actuelle. Il n'est pas douteux qu'une investigation systématique d'un grand nombre de ces cas apporterait une contribution susceptible de donner des bases plus objectives à la phylogénie.

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PURPLE DWARF, AN UNDESCRIBED POTATO DISEASE, IN ALBERTA¹

BY G. B. SANFORD² AND S. B. CLAY³

Abstract

The characteristic symptoms of what seems a new disease of potato, observed in Alberta, have been outlined, certain observations and preliminary experiments reported, and the name "purple dwarf" suggested for it.

Purple dwarf is perpetuated through the tubers, and the symptoms on plants from diseased tubers either appear immediately the plant emerges from the ground or apparently fail to become recognizable during the remainder of the season.

Apical growth is checked, the entire plant becomes stunted, the newer leaves develop a purplish hue along their margins and curl upwards, the phloem of the entire plant is disorganized, and a well developed, brown, dendritic necrosis extends from proximal to distal ends of the tuber. First the roots, then the stolons, and later the base of the stem, become brown and soon decay. Usually the central pith in the tuber and upper stem remains normal.

Purple dwarf was transmitted to healthy plants by grafting, a result which suggests that the causal agent is a virus.

Introduction

A potato disease, apparently different from any yet described, has been observed in Alberta during the past decade. However, the data are not yet adequate to indicate whether this disease is increasing. Specimens have been found in many potato fields in the southern, central, and north-central sections of Alberta.

The disease seems to develop equally well in all districts. The number of affected plants rarely exceeds 1%; many fields appear to be free from the disease. On the other hand, there has been recorded a few special cases in which the proportion of diseased plants is as high as 3 to 5%. All potato varieties seem to be susceptible. Although the malady has not yet seriously reduced yield, its early recognition and control is of much importance.

Following the preliminary observations, a more extensive study of the malady was begun in 1938, and sufficient data is now available to justify a preliminary description of this disease, for which the name "purple dwarf" is proposed.

Description

EXTERNAL PLANT SYMPTOMS

Symptoms of purple dwarf on the potato are, in some respects, similar to those listed by Barrus and Chupp (2), Muncie (3), and by Walker and Larson (5) for yellow dwarf, and also to those listed by Orton and Hill (4) for the blue stem disease of potatoes.

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Although the external plant symptoms vary considerably, they seem to be within a rather well defined pattern. Usually purple dwarf plants can be easily recognized as they emerge from affected sets (seed pieces) planted under field or greenhouse conditions. The entire plant is more or less stunted, rigid, rather brittle, and often of a darker green hue than healthy plants (Plate I, *A*). Frequently the sprouts from severely affected sets do not reach the surface, for they may remain abortive and eventually die. General stunting and distortion of the plant and early development of purple colour, especially on the margins of the apical leaves, are constant symptoms under field conditions. Plate I, *B*, illustrates the appearance of a plant in which the symptoms developed more slowly than usual.

The young leaves are dwarfed, typically wrinkled, and cupped. A definite, but usually not extensive, rosette growth of foliage is common at the upper nodes, and this may or may not be accompanied later by tuberous swellings of axillary shoots or stunted leaves. The petioles of the primary leaves usually are dwarfed and stiff, often slightly swollen, and curved upwards. The main stems do not assume a zig-zag direction or characteristically swell at the nodes. Some of the lower leaves of the plant may eventually become slightly purplish or yellowish along the margins and roll inward, but when this stage is reached, chlorotic areas between the veins of the leaves soon appear and death of the foliage and of the characteristic dark green stalks gradually proceeds. Sudden wilting of the plant does not occur, except when severe stem rot, caused by other agencies, develops.

All the underground parts of a plant showing early symptoms of purple dwarf are at first apparently normal, but soon the older roots, then the stolons, and finally the epidermis of the lower stem, turn brown and decay. This general browning begins at the extreme base of the stem and proceeds outward through the roots and stolons and upward through the stem (Plate II, *A*). The outer portion of these brown roots and stolons may be slipped off with ease. Plants from infected sets that show definite purple dwarf symptoms rarely yield tubers more than one inch in diameter; usually only a very few small ones, or none, develop. These tubers, which always show a severe necrosis, are not characteristically cracked or off-type, as is often the case in yellow dwarf. The stolons and tubers do not produce secondary growth. The parent set usually remains sound throughout the season.

Internal Plant Symptoms

Pith

Under field conditions, the pith of the stem of purple dwarf plants is at first normal to dark green in colour, and sound. It remains in this state until the disease is well advanced. However, after a few days a general disorganization may begin at the base of the stem, as a result of invasion by bacteria and fungi. This decay gradually extends upward in the pith for a distance of from two to three inches (Plate III, *A*). An occasional small local rusty area in the pith of the stem may occur in certain badly diseased

plants, but in most instances it is absent. The pith in the stolons and tubers is apparently normal. Therefore, in contrast to yellow dwarf (5), pith necrosis alone is not a reliable diagnostic symptom of purple dwarf.

Xylem

Examination under a microscope of a cross-section of the stem of a plant having typical purple dwarf symptoms indicates that the vessels are free from obstruction and apparently normal (Plate III, *A* and *C*). However, in a few instances where affected phloem strands were adjacent to these vessels, a slight discoloration of the inside wall of certain vessels occurred. This generally normal condition of the xylem is maintained throughout the entire plant. On the other hand, if a longitudinal section of the stem (Plate III, *E*) is viewed with the unaided eye, the general vascular area is seen to be more prominent and more discoloured than in uninfected stems, increasingly so toward the base. The discoloration mentioned occurs in the phloem strands on both sides of the xylem (Plate III, *C*, *G*, and *H*).

Phloem

The phloem throughout stems, stolons, and roots of purple dwarf plants is discoloured and often plugged with a substance that stains deeply with Sudan III (Plate III). Occasionally some of the sieve tubes are distorted and large. The walls of the phloem parenchyma also become thickened and stain with Sudan III. In young tubers the secondary phloem groups adjoining the storage parenchyma are usually more prominent than the outer circle of phloem groups adjacent to and in the cortical tissue. The necrotic phloem strands throughout the broad band of storage parenchyma in tubers are also more or less plugged and brown. Thus, in cross or longitudinal section, it is these necrotic phloem strands that form the typical netted pattern in affected tubers. (Plate III, *B* and *F*). As a rule the starch grains in the immediate vicinity of these necrotic strands, in tubers, are small or absent.

Another easily recognized symptom of purple dwarf, is a marked browning of the exterior of the vascular cylinder in the lower stem, stolons, and roots. This discoloration, which is revealed when the cortical and epidermal tissues are peeled back, may often resemble the browning associated with *Fusarium* or *Verticillium* wilt. In cases where the disease originates from a diseased set, the discoloration begins at the base of the stem and proceeds upward in the stem and outward in the roots and stolons. In a few days this change is accompanied by the accumulation of excess moisture. At this stage, bacteria are present, but fungi are rarely isolated. The brown exterior of the central stele of affected plants is illustrated in Plate II, *B*. Further, the browning just described is most pronounced at the nodes where the roots and stolons leave the stem (Plate II, *B*). This might be expected in view of the greater development of the phloem at this point (1).

Cortex

The cortex of the lower stem, stolons, and roots retains its natural appearance for a short time after browning of the phloem occurs, then becomes discoloured and finally disintegrates. In the roots, complete disintegration of the endodermis and cortex may take place relatively early, but in the stolons, and occasionally in the lower stem, this is more gradual.

PURPLE DWARF TRANSMITTED THROUGH TUBERS

The results from experiments and extensive field observations leave no doubt in the minds of the authors that purple dwarf is transmitted through affected tubers. To date all definite purple dwarf plants appearing in commercial potato fields have originated from tubers that carried the purple dwarf disease. A few of the typical examples observed will be cited to illustrate this connection.

In one plot of about one-third acre, planted for foundation material with selected stock by the tuber-unit method, there were three units in which purple dwarf developed. All four sets in one unit produced typical purple dwarf plants, each with different degrees of severity (Plate IV, A). This is not uncommon. In the second unit three plants were healthy and one had purple dwarf. The third unit produced three purple dwarf plants and a healthy one. This apparently healthy plant, which was transplanted to the laboratory field plots, did not develop purple dwarf symptoms. None of the sets of the three affected units had internal net necrosis.

In a nearby commercial field (approximately two acres) of Netted Gem potatoes, 11 typical purple dwarf plants were observed during early July. The sets of these plants did not have internal net necrosis. Also, no other plants in either of the two fields mentioned developed purple dwarf symptoms during the season.

In another foundation plot of about one-half acre in size, and located 30 miles distant, there were eight tuber units in which no plants emerged, and one in which four plants emerged and all had purple dwarf symptoms, but no tubers were produced. The plants in all other unaffected units in the plot were apparently normal throughout the season. Internal net necrosis was apparently absent in the sets that produced the purple dwarf unit just mentioned, and also absent in the sets of all but two units of those that failed to produce plants.

Many other cases could be mentioned to illustrate that the purple dwarf plants, which appear early in potato fields, originate from random tubers that carry this disease. Also, the available evidence indicates that in many instances the sets that produce purple dwarf plants are apparently free from internal net necrosis. On the other hand, it might be expected that some net necrosis would be found in the parent tubers, because whenever tubers are formed on plants with definite purple dwarf symptoms they apparently always develop severe internal necrosis.

DISEASE DEVELOPS EARLY

Present evidence indicates that either the disease shows up early in a pronounced and definite form, as soon as the plants from affected tubers emerge from the ground, or not at any time later during the season. In view of this situation one suspects that the initial symptoms of purple dwarf, from possible field infection later in the season, must be slight or masked. Obviously the identification of these initial symptoms is an important factor affecting control measures.

Experimental Results

TRANSMISSION OF PURPLE DWARF

During the present season, attempts were made to transmit purple dwarf to healthy potato, tobacco, and tomato plants. One method was to place in the stem of a healthy plant a short length of a petiole, with leaflet attached, taken from an affected plant. Another method was to transfer a thin disc from the stem of an affected plant to an incision made in the stem of a healthy plant, or to one made near the semi-dormant eye of a healthy potato set. Also, a thin slice was aseptically removed from the eye of potato sets that had produced purple dwarf plants, and buried near the eye of a healthy set. The grafts were waxed to prevent loss of moisture. These various experiments, and results obtained from them to date are listed in Table I.

The data in Table I indicate that in potatoes purple dwarf can be transferred by inserting a short length of a petiole from an affected plant into the stem of a healthy plant. One of these purple dwarf plants produced by grafting is illustrated in Plate IV, B. The small tubers had severe internal

TABLE I
RESULTS OF INSERTING PURPLE DWARF TISSUE INTO HEALTHY POTATO, TOMATO,
AND TOBACCO PLANTS

Expt.	Source of purple dwarf tissue		Grafts				Number of plants, %		Number of checks
	Variety	Origin	Variety	Place	Plants	Date	Positive ¹	Negative	
1	Warba	Petiole	Warba	Stem	13	6/7	7	6	13
2	Warba	Petiole	Ohio	Stem	6	6/7	0	6	6
3	Warba	Base ²	Ohio	Set ³	15	6/7	0	15	10
4	Gem	Base	Warba	Stem	9	12/7	2	7	9
5	Gem	Base	Warba	Set	22	17/7	0	22	10
6	Gem	Old set	Warba	Set	9	17/7	0	9	10
7	Warba ⁴	Petiole	Tomato	Stem	10	7/8	0	5	5
8	Warba ⁴	Petiole	Tobacco	Stem	4	7/8	3 ⁵	1?	4

¹ Typical purple dwarf symptoms.

² Base of stem.

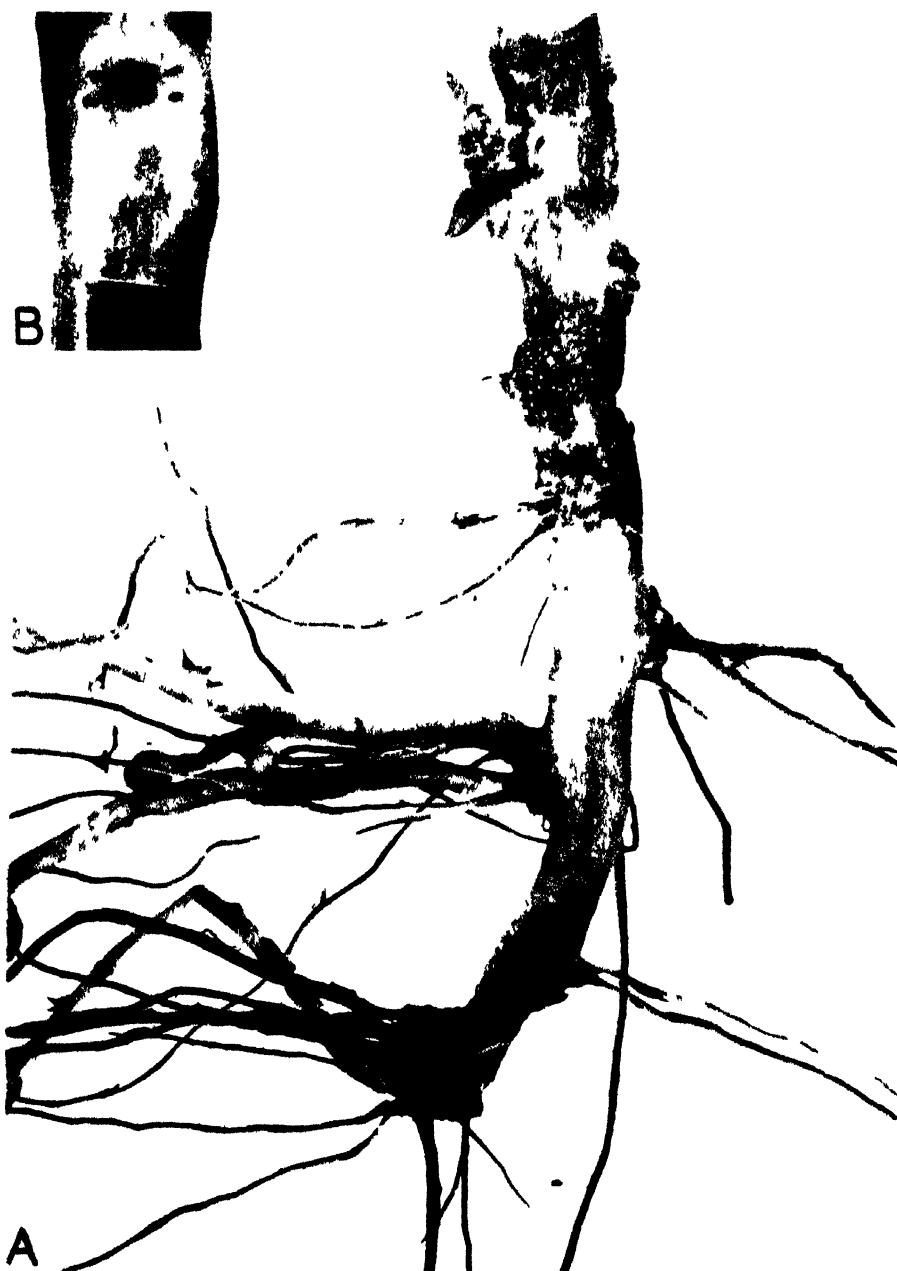
³ Tissue inserted adjacent to eye.

⁴ From Experiment No. 1.

⁵ Plants stunted, leaves mottled, and area surrounding veins dark green.

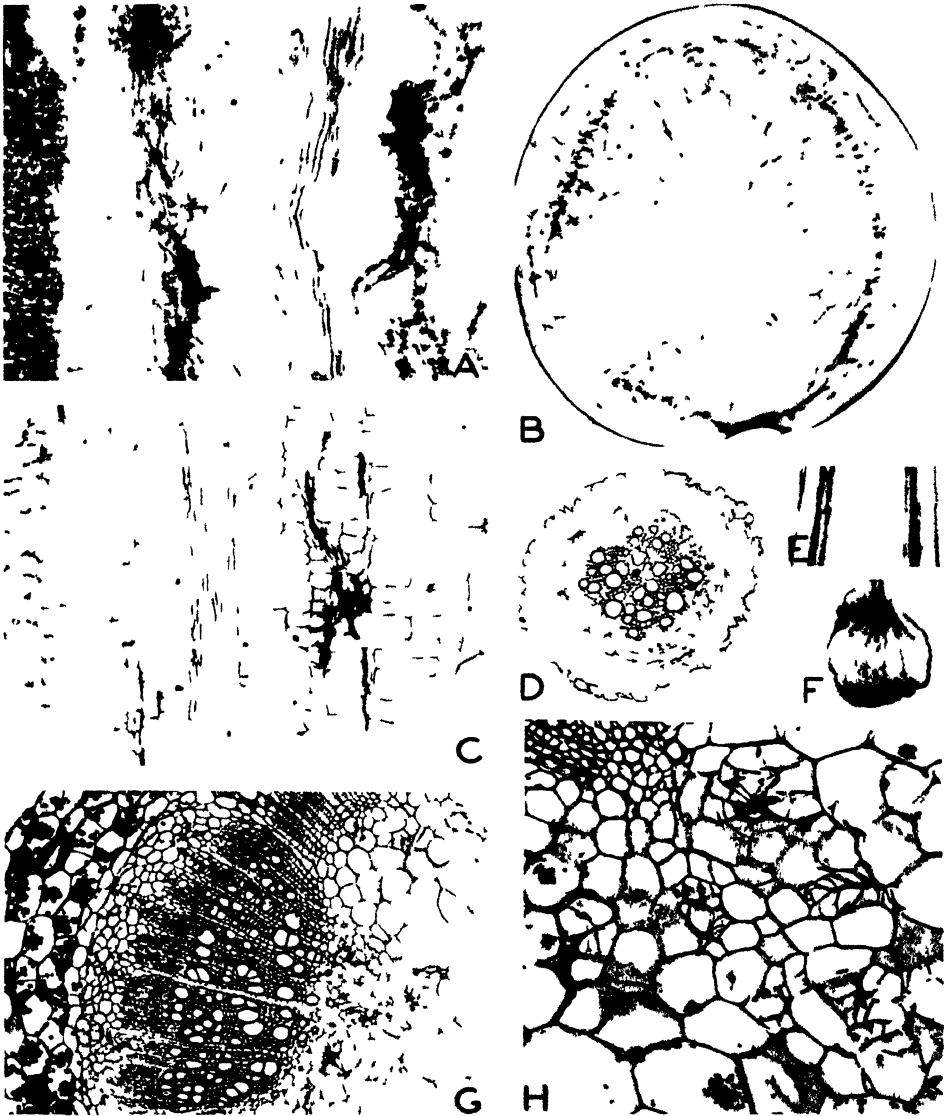


A. Typical purple dwarf symptoms soon after emergence. The lower set of roots are



A Lower stem, roots, stolons, and small tubers of a purple dwarf plant Some roots and stolons are more affected than others and the end of the stem is rotting

B. Brown interior of roots and stolons at node, also the exposed brown central stele below.



A Longitudinal section of tuber Note left to right normal periderm and cortex, affected external phloem, normal xylem, and diseased internal phloem

B Cross section of tuber in A Note normal central pith and wide distribution of affected phloem groups

C Longitudinal section of stem at ground level showing diseased phloem elements and normal cortex and xylem Note outer phloem groups are less prominent than the inner ones.

D Cross section of root of purple dwarf plant showing affected phloem and disorganization of adjacent cells

E and F Longitudinal sections of stem and tuber of purple dwarf plant showing (E) brown vascular region in stem and (F) brown phloem strands throughout young tuber with massing at stem end

G Cross section of stem in C Note internal phloem groups are more prominent than external phloem groups adjoining cortex

H Affected groups of inner phloem in G, magnified



A. All four plants of this unit have typical purple dwarf symptoms. Note differences in development and also the healthy unit in background.

B One of the plants which developed typical purple dwarf symptoms as a result of grafting experiments. (See Table I and context)

net necrosis. Although slightly more than 50% of the grafts were successful in Experiment 1, and only 22% in Experiment 4, it is now known that a much greater degree of success is possible.

It is important to note that the disease was apparently not transferred by inserting tissue from potato sets that had produced typical purple dwarf plants into the eyes of healthy sets. Negative results were also obtained with stem tissue. However, there is the possibility that purple dwarf will develop from the progeny of the plants when planted next season.

So far, negative results only were obtained from the grafts made on tomato plants. Further attempts will be made. However, from the grafts on tobacco (*Nicotiana tabacum*) were produced stunted plants with mosaic on the foliage. The effect was definite and uniform for each plant.

The results of the experiments listed in Table I indicate that much more can be learned, by continuing this type of work, about the nature and behavior of purple dwarf, especially whether the potato plant can carry the disease without developing recognizable symptoms in the field.

Etiology

The virus nature of purple dwarf is suggested because the disease originates from affected tubers, the tissue of which yields neither bacteria nor fungi when cultured on potato dextrose agar, and also by the fact that typical purple dwarf symptoms have been reproduced experimentally within approximately 30 days by grafting diseased tissue into healthy plants. At present no information is available regarding the possible role of insects in spreading purple dwarf. Apparently the disease must be spread by some agent to a few plants each year, and definite symptoms fail to appear immediately in such cases, or it develops in some obscure manner in the tubers subsequent to their harvest. Possibly contact between the foliage of purple dwarf and healthy plants in the field would be sufficient to transfer the malady, but no evidence of this has been observed to date. At any rate, plants that develop definite purple dwarf symptoms from diseased tubers either die before new tubers are formed, or, if any develop, they are small, severely netted, and unable to produce sprouts that emerge above ground. This may explain why the malady does not increase more rapidly. It is hoped that results obtained from grafting diseased tissue into healthy plants will throw necessary light on some of the obscure points mentioned.

Discussion

Although many aspects of the purple dwarf disease remain to be clarified, the more important distinguishing characteristics of the malady can be presented at this time.

In several ways, purple dwarf seems to be essentially different from yellow dwarf (Barrus and Chupp (2), Muncie (3), and Walker and Larson (5)). For example, there is no prompt die-back from the tips of affected plants.

Also, the tubers are uniformly small, or do not develop, and they are not misshapen. Affected plants die rather slowly and do not wilt suddenly. Further, pith necrosis is by no means a diagnostic feature of purple dwarf, because very often it is not present in the stem, and apparently absent in the tuber. Moreover, internal net necrosis in purple dwarf tubers is a typical symptom that is confined to the phloem throughout the tuber and does not consist of other groups of disorganized cells. Finally, the foliage of affected plants develops typical purplish pigments, seldom yellowish ones.

On the other hand, purple dwarf and yellow dwarf are similar in certain respects, because both diseases are apparently of a virus nature and carried by the tubers. Moreover, if healthy plants become infected later during the growing season, they apparently fail to develop easily recognizable purple dwarf symptoms either on the foliage or in the tubers. Further research will show whether this can be proved to be the case.

Purple dwarf also appears to be different from the blue stem disease described by Orton and Hill (4). In contrast with purple dwarf, definite symptoms of blue stem apparently develop quickly on normal plants at any stage from the beginning of tuberization to maturity, and the plants usually wilt within two weeks. Another important difference is that the blue stem disease is not perpetuated through the tubers, and that tubers of marketable size may be produced if the infection is not too early. Further, infection of plants under field conditions seems to be general and the blue stem symptoms unmistakable. Finally, the dendritic necrosis seems usually confined to the first half or quarter of the stem end of the tuber, but in purple dwarf it is characteristic for this necrosis to permeate the entire length of the tuber.

Acknowledgment

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STUDIES ON THE PERENNIAL RUST *PUCCINIA MINUSSENSIS*¹

BY A. M. BROWN²

Abstract

The permanent infection of rust-free *Lactuca pulchella* plants by *Puccinia minusensis* may be initiated by inoculating the primary leaves of seedlings, or the rhizome buds of older plants, with either aeciospores or urediospores, or by packing teliospores into the leaf axils of mature plants and subsequently using the infected nodal portions as propagation cuttings. When a primary leaf of a seedling becomes infected, the binucleate mycelium progresses along the midrib and petiole to the stem and advances down it into the rhizomes but, before reaching the terminal buds of the rhizomes, its nuclear condition changes, as only uninucleate mycelium has been observed in the bud scales. Infected rhizome buds develop into young shoots that usually first produce pycnia, although, in cases of delayed mycelial development, telia appear on the more mature plant followed by pycnia on subsequent new axillary growth. The perennial mycelium seems to alternate from the binucleate to the uninucleate condition and vice versa, depending apparently on the maturity of the host tissue involved and the food supply available to the fungus.

Introduction

In the past, perennial rusts of plants have attracted the attention of several investigators. Plowright (6) appears to have been the first to successfully initiate permanent infection in a host plant. On sowing teliospores of *Endophyllum Euphorbiae* DC. on very young plants of *Euphorbia amygdaloides*, he noted that the subsequent infection progressed along the leaf petioles to the stems, where the mycelium could be found "in the pith and inner bark." The mycelium then invaded the subterranean part of the host. Describing further development, he states: "The foliage and shoots sent up by it in the following year, are pervaded by the perennial mycelium, and produce aecia abundantly during the spring, but the late summer and autumn foliage are somewhat shorter. The next vernal foliage is, however, aecidiiferous." Plowright (6) failed, however, to secure the permanent infection of old plants, apparently because defoliation occurred before the mycelium reached the stems.

Dodge (2), in his investigations on *Gymnoconia peckiana* (Howe) Trotter and *Kunkelia nitens* (Schw.) Arth., succeeded in permanently infecting *Rubus* species by inoculating emerging shoots. Older shoots, however, when inoculated near the top, always failed to become permanently infected.

From a cytological study of three rusts, all of which he regarded as perennial, Olive (5) concluded that the uninucleate mycelium and the binucleate mycelium of these rusts were of independent origin. Kursanov (3), in his

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interpretation of the nuclear behaviour of *Trachyspora Alchemillae* Fckl., seems to have accepted Olive's explanation, whereas Lindfors (4) concluded that all the mycelium of this rust was uninucleate and that binucleate cells arose as a result of cell fusion or nuclear migration.

Among the perennial rusts found in Western Canada is *Puccinia minussensis* Thum., which occurs abundantly on *Lactuca pulchella* (Pursh) DC. This rust is euautoecious. The aecia are reported to occur on a perennial mycelium (1), the uredia and telia presumably arising as a result of secondary infections. Field observations made at Winnipeg by the writer indicated, however, that uredia and telia might also arise from perennial mycelium, and this observation led to the initiation of experiments dealing with the life cycle and nuclear behaviour of this rust.

Experimental Procedure and Results

Early in June, 1932, naturally infected plants of *L. pulchella* were transplanted from the field to the greenhouse. At this time pycnia had developed on the leaves and the infected plants gave off a sweetish odour that was noticeable, especially in damp weather, at a distance of several feet. As the pycnium-bearing leaves aged, aecia developed along the midribs, forming progressively from the petioles to the tips of the leaves. Unlike the pycnia, which covered the whole leaf surface, the aecia were largely confined to the midribs, although both pycnia and aecia occurred sparingly on the stems. In several aecia, the aeciospores were suppressed and teliospores developed in their place (Fig. 1), whereas, on some leaves, telia followed pycnia directly (Fig. 2). In the latter case, the telia appeared on the leaf blades and were not confined to any particular location, as the aecia had been. Sometimes uredia, interspersed among the aecia, appeared along the midribs, but urediospores did not displace the aeciospores as did the teliospores just mentioned.

When the infected plants died back in October, the rhizomes were removed from them and reset singly in pots. In January, 1933, new shoots emerged in these pots and a majority of them, when from three to four inches high, produced pycnia and then aecia in due course. On some of the shoots developing pycnia, the short-cycling tendencies referred to above were encountered. On two of the plants arising from the reset rhizomes, however, pycnia did not develop but, when the plants reached maturity, telia were produced on the lower half of the stems. These stems were then cut back, leaving only the parts on which the telial lesions were present. Three or four weeks later, axillary growth developed and on this new growth pycnia and aecia developed in sequence.

The results secured in the experiments just described indicated that the perennial mycelium changed from the uninucleate to the binucleate condition and vice versa, within the host. In order to examine this nuclear phenomenon more thoroughly, a series of experiments were initiated.

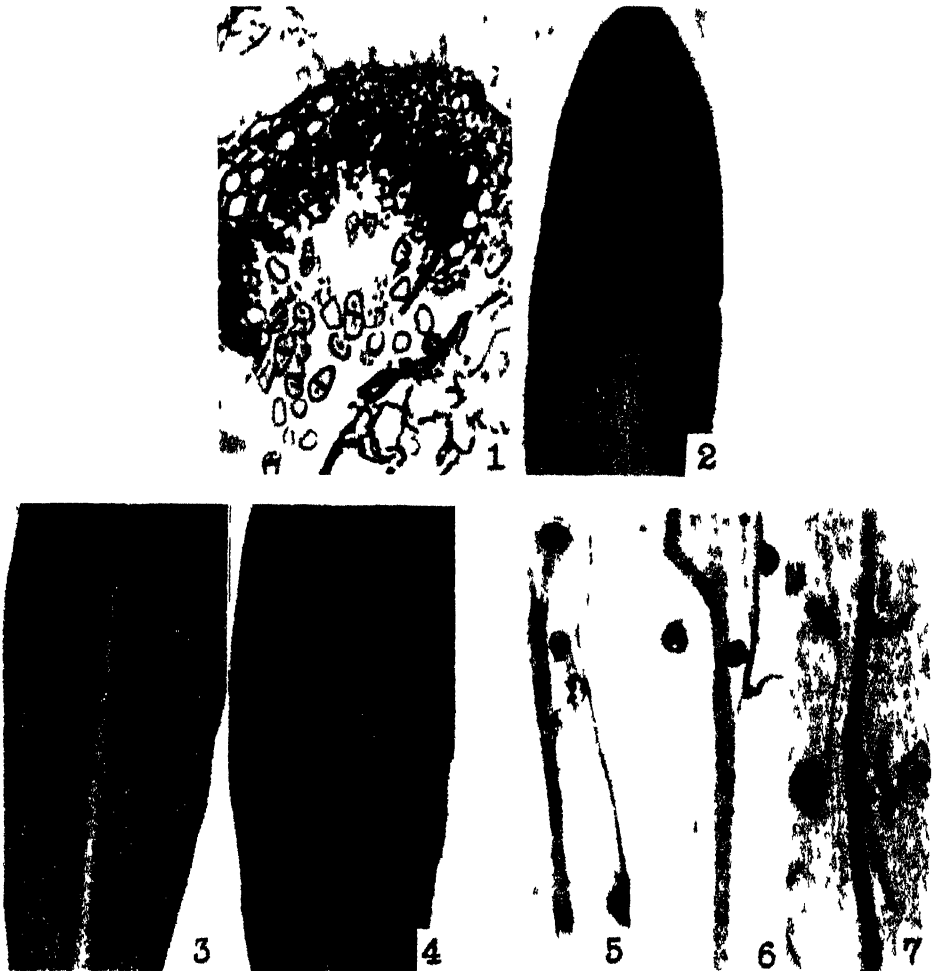


FIG. 1 Photomicrograph of a section of an aecium cup. Teliospores are present instead of aeciospores. $\times 350$

FIG. 2 Infection in which telia occurred directly after pycnia. The pycnia are still seen following the leaf veins. About natural size.

FIG. 3 A uredial infection on *Lactuca pulchella*. After invading the midrib it is extending along it in both directions.

FIG. 4 The same infection 28 days later. Compare rate of growth with that of the localized infection of the same age on the right.

FIG. 5 Photomicrograph showing paired and single nuclei in the mycelium of a rhizome, section cut from the area adjacent to the root crown. $\times 500$

FIG. 6 Photomicrograph showing mostly single nuclei in a section cut from a rhizome near the terminal bud. $\times 600$

FIG. 7 Photomicrograph showing uninucleate mycelium in the scales from the terminal bud of the rhizome. $\times 900$

Inoculation of Seedlings

Ten seedling *L. pulchella* plants grown in the greenhouse were inoculated in August, 1933, by sowing urediospores on the primary leaves, so that the resulting infections would occur on or near the leaf midribs and adjacent to the petioles. Two weeks later several isolated uredial infections developed close to the midribs and eventually invaded them. The uredial infections after invading the midribs rapidly expanded and in due time reached the stems by way of the petioles. One of these infections was closely observed and was twice photographed. At the time of the first photograph (Fig. 3) it was 25 mm. long; at the time of the second (Fig. 4), 28 days later, it measured 45 mm. Its growth rate, which was therefore 0.72 mm. in each 24 hr., can be compared to that of the isolated infection of the same age occurring on the right of the midrib.

The leaves on which midrib infections developed were kept in a vigorous state of growth by pinching out the terminal bud of the plants bearing them. Late in December, when all the midrib infections had invaded, or almost invaded, the stems, the hosts were placed beneath a bench in the greenhouse where they remained until February, 1934. During this time the temperature of the greenhouse was maintained close to 13° C. About the end of February the pots were taken from this greenhouse. A large proportion of the original stems in each pot remained green. Two of the pots were placed in a warm greenhouse. From the remaining eight pots the rhizomes were removed and reset singly, the stems and other plant parts being discarded.

In March, new axillary growth appeared on the stems in the two undisturbed pots, and this new growth produced, for the most part, pycnia and aecia in sequence. Occasionally teliospores appeared in place of aeciospores in some aecia, or telia occurred directly after pycnia. From the reset rhizomes new shoots emerged in May, and a majority of them produced pycnia. On one shoot, however, telia developed directly after pycnia, and, on another shoot, uredia occurred, interspersed with aecia. Two shoots arising from reset rhizomes continued without visible rust infection until they approached maturity; then telia developed on the lower half of the stems. The stems were then cut back to a point just above the uppermost telial sorus. This induced new axillary growth on which pycnia and aecia were produced in sequence, but with teliospores instead of aeciospores in some aecial cups.

From these results it seemed clear that the perennial mycelium of *Puccinia minusensis* could, under certain conditions, be initiated by urediospore inoculation of its host. The initial mycelium of the infection would therefore be binucleate; but, to produce pycnia in the new axillary growth of the host, the binucleate condition of the mycelium must have undergone change. Apparently, however, the change from the binucleate to the uninucleate condition did not always occur in every plant, as telia, unaccompanied by pycnia or aecia, developed on the new growth of several plants.

The unusual nuclear behaviour just mentioned was investigated more fully by repeating the experiments described above during the years 1934 to 1937. During this period, 50 seedling plants were inoculated with aeciospores so that the ensuing infections would readily invade the stems by way of the midribs and petioles. The inoculated plants were treated in a manner similar to that adopted in the previously described experiments, and the results secured were identical with those already given. In addition, 10 uninoculated plants were maintained as checks, but they remained free from infection. Moreover, repeated attempts to establish the systemic mycelium of this rust in maturing blue lettuce plants by aeciospore or urediospore inoculation failed, owing probably to defoliation before the infections could invade the midribs, petioles, and stems.

Inoculation of Rhizome Buds

An examination of the rhizomes of older plants showed that well developed stomata were present on the scales of the terminal buds. As the bud scales could be separated without causing injury, aeciospores were inserted between the scales of 50 buds on the rhizomes of rust-free plants. Ten uninoculated buds on other rhizomes were kept as checks. The rhizomes bearing the inoculated buds were kept in a moist chamber for two days before they were reset singly in pots. The controls were treated similarly. Thereafter the pots were kept under ordinary greenhouse conditions. Two months later new shoots appeared above ground in all the pots; when they were from two to three inches in height, 47 out of 50 of the shoots originating from inoculated buds produced pycnia whereas the shoots from the uninoculated buds remained rust-free.

As a rule, aecia followed pycnia, but, as in the former experiments, teliospores sometimes occurred instead of aeciospores, and uredia appeared in association with aecia. However, three shoots from inoculated buds continued without visible rust infection until they were almost mature plants; at that time telia occurred on the lower half of the stems. As in the former experiments, the stems were cut back to just above the telial sori; on subsequent axillary growth were produced pycnia and aecia, as had happened in the previous experiment.

From these results it is seen that the inoculation of rhizome buds with aeciospores gives results identical with those obtained by inoculating leaves of seedling plants with aeciospores or urediospores. In passing, it may be observed that by inoculating the rhizome buds the time of experimentation was reduced by approximately one-half, as pycnia developed within two months, whereas, with seedling inoculation, pycnia developed after four months.

Inoculation of Mature Plants

In these experiments, many attempts were made to establish systemic infection by inoculating mature plants with aeciospores or urediospores, but always with negative results. This was the experience of Plowright (6) with *Endophyllum Euphorbiae* and Dodge (2) with *Gymnoconia peckiana* and

Kunkelia nitens. Positive results, however, were eventually obtained by one method. Teliospores were packed into the leaf axils of plants almost mature. Two weeks later, wart-like swellings appeared on the stems just above the nodes, where teliospores had been in contact with them, and on these swellings telial sori developed. By wrapping the infected areas in moist cotton wool, adventitious roots were induced to develop at the nodes, and, from such nodes, cuttings were obtained that eventually gave rise to permanently infected plants. Just how the infections were brought about is not known, but it is presumed that sporidia from germinating teliospores initiated them. The variations previously encountered in the life cycle of the organism were also observed in this experiment.

Nuclear Behaviour

An examination of plants in which permanent infection was induced through inoculation of the leaves by aeciospores or urediospores, showed that the binucleate mycelium invaded the stems from the petioles and that the mycelium remained binucleate until it invaded the root crown. From there the mycelium penetrated the rhizomes and, in them, evidently changed to the uninucleate condition. At any rate, microtome sections cut from rhizomes near the root crown showed large mycelial cells, each containing several paired and a few single nuclei (Fig. 5). Between this area and the terminal bud of the rhizome each mycelial cell, still large, contained few paired and many single nuclei (Fig. 6), but, in the bud scales, only uninucleate cells were found (Fig. 7).

A similar nuclear condition was observed in sections cut from axillary shoots arising from stems that had produced only telia and had been cut back to induce new growth. On the new shoot, pycnia first appeared on the second pair of new leaves as they unfolded. The mycelium in the basal tissue of the shoot was binucleate but uninucleate in the terminal bud, and obviously so in the leaves producing pycnia. A change in the nuclear condition of the mycelium must therefore have occurred in the lower part of the shoot. The aecial primordium was uninucleate. The return from the uninucleate to the binucleate condition occurred at the base of the aecium.

From the experimental results, it appears evident that the nuclear condition of the mycelium is closely related to the age of the tissue in which it is present and is apparently dependent, as suggested by Whetzel, Jackson, and Mains (7), on the maturity of the host tissue and the supply of food available to the fungus.

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THE "LEAF GAP" IN *EQUISETUM*¹BY RUTH MOORE²

Abstract

A previously undescribed feature of the structure of the stem in *Equisetum* has been found. This conforms more nearly with a true leaf gap than any other feature of *Equisetum* hitherto so considered. Its interpretation and bearing on the classification of the vascular plants into Lycopsidea and Pteropsida are discussed.

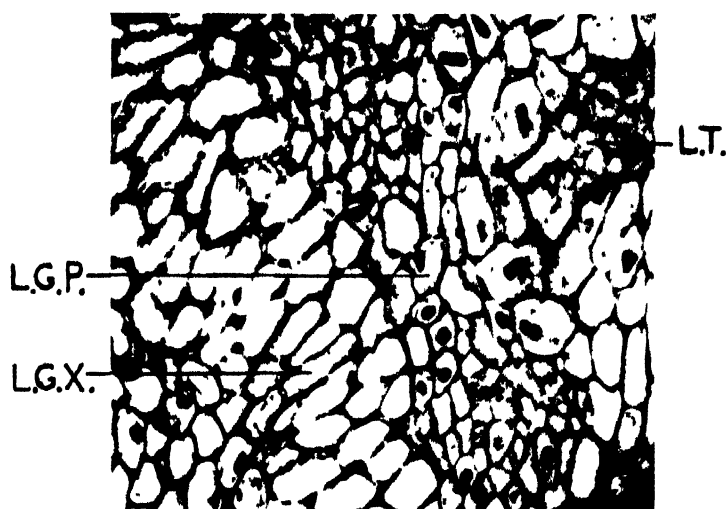
Introduction

In 1899, Jeffrey (4) proposed a division of the vascular plants into two groups, the Lycopsidea and the Pteropsida. The Lycopsidea comprised the vascular forms below the ferns and thus included the genus *Equisetum*. Its members were characterized, among other features, as microphyllous plants that were without foliar gaps. The Pteropsida, on the other hand, were considered fundamentally megaphyllous, with a well developed parenchymatous leaf gap in the cauline vascular tissue immediately above the outgoing leaf trace, leaving the parenchyma of the medulla and cortex in direct connection with each other. The classification was for a time widely accepted and the belief is still general that a leaf gap is not present in *Equisetum* (3, p. 99) despite the fact that certain features in the anatomy of the plant have been held by various writers to represent this gap. For example, in the stem the large parenchyma lacunae between the forking vascular strands have been interpreted as foliar gaps. However, since vascular tissue of the stem usually intervenes between trace and lacuna, and since in those forms that have a continuous endodermis, this endodermis remains unbroken, the lacunae do not fulfil one of the basic requirements of a leaf gap in that they are not in direct contact with the leaf trace and they do not place the pith and cortex in connection. In the cone, too, Browne (1) has suggested that the parenchymatous strands between the conducting tissues of the axis are leaf gaps, but here also nodal xylem may separate trace and parenchyma. Cases where this is not true and the parenchyma lies immediately above the sporangiophore trace could be explained not as a primary, but as a secondary feature resulting from reduced xylem development. Moreover, as in the stem, the endodermis, in certain forms at least, separates the medulla and cortex. Johnson (5) appears to be the only one to recognize the importance of this feature. In his study of the cones of *E. scirpoides*, he observed the reduction in supranodal wood bringing the trace and the parenchyma together and mentioned the fact that the presence of the unbroken endodermis across the gap was, in his opinion, the only indication that it was not a true foliar gap. In addition, these parenchyma strands are probably homologous with the lacunae of

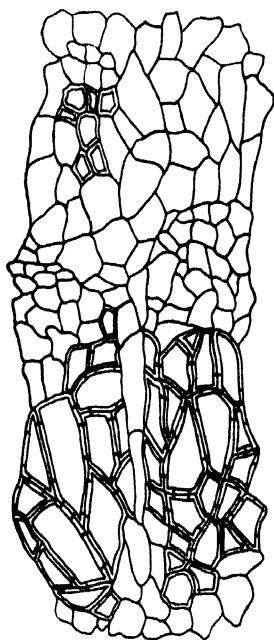
¹ Manuscript received December 3, 1940.

Contribution from the Department of Botany, University of Toronto, with assistance from the National Research Council of Canada.

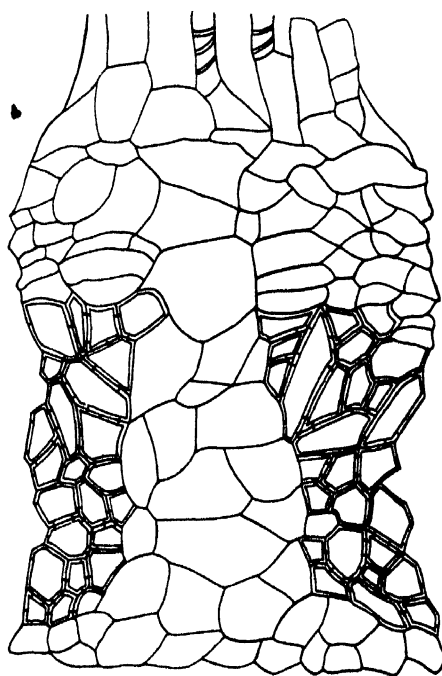
² Graduate student, University of Toronto.



A



B



C

FIG. 1. Transverse sections of the "leaf gap" in the stem of *Equisetum*. A: *E. sylvaticum*; L.G.P., "leaf gap" in the phloem; L.G.X., "leaf gap" in the xylem; L.T., leaf trace. $\times 280$. B: Vegetative stem of *E. arvense*. $\times 300$. C: Fertile stem of *E. arvense*. $\times 300$.

the stem which, as previously noted, cannot be considered foliar gaps. Of course it might be argued by those who believe the cone to be a conservative region that the apparent foliar gap that may occur there when the xylem above the trace is undeveloped is a primary character and that the supranodal wood intervening between the trace and the parenchyma in both stem and cone is a later development obscuring the true nature of the parenchyma as a foliar gap. However, another feature found by the writer in *Equisetum*, may correspond more completely with the Pteropsid leaf gap.

Description

Transverse sections cut through the node of the *Equisetum* stem show frequently, although by no means always, a band of parenchyma one to two or three cells wide, and as many high, crossing the conducting tissue immediately above the departing leaf trace (Fig. 1A). This band is closed over by vascular elements that separate it from the large lacuna above. It is not illustrated here in longitudinal section, but it may be described as lying above any of the leaf traces of Fig. 2C, very similar, except for its smaller development, to the parenchyma island associated with the trace at the extreme right of Fig. 2B. In some cases the pith is in direct contact with the parenchyma of the cortex through the narrow band; in others, the endodermis is continuous so that no actual connection is made. In those species in which it is present the internal endodermis is always unbroken. The parenchymatous band has been observed in seven species examined:— *E. arvense* L., *E. giganteum* L., *E. limosum* L., *E. palustre* L., *E. praealtum* Raf., *E. scirpoides* Michx., and *E. sylvaticum* L. It is particularly well marked in the vegetative nodes of the fertile stem of *E. arvense* (Fig. 1C) where, in contrast with the sterile stem (Fig. 1B), the parenchyma cells are somewhat more numerous and are not radially elongated. In *E. palustre* and *E. scirpoides*, both of which are species with somewhat reduced xylem, the nodal xylem may occasionally fail to close over the gap whereas in *E. praealtum*, sometimes also in *E. arvense*, the number of parenchyma cells may be increased so that the band extends down the sides and underneath the leaf trace. Apparently a somewhat similar condition has been observed by Browne (2) in *E. kansanum* where she notes that the leaf trace passes through a "parenchymatous gully". Further study of the extent of these variations in the different species would be necessary to determine whether they have taxonomic significance.

Cones of six of the above mentioned species were also studied. No evidence of a gap was found at any of the fertile nodes. This was true of both normal and markedly abnormal cones. Only in the case of abnormal and infertile annuli that were leaf-like and vascularized were there any indications of its presence.

Discussion

It appears that the only previous reference made to this parenchyma band is Browne's mention of the parenchyma gully in *E. kansanum*. The band differs from the commonly accepted type of Pteropsid leaf gap in its very

small size, in the irregularity of its occurrence and its form, and in the frequent presence of the unbroken endodermis. However, as a parenchyma band passing through cauline vascular tissue directly above a leaf trace, at times connecting pith and cortex, it appears to justify consideration as a leaf gap, in which light it is here proposed to discuss it. The only other interpretation that seems possible is that it might be storage parenchyma associated with the assimilatory function of the leaf; in *Equisetum*, however, the leaf is of minor importance as a photosynthetic organ. In the fertile shoot of *E. arvense* (which lacks chlorenchyma tissue) leaf base storage would be very unlikely, but here the gap is particularly well developed. In addition, this fertile stem has little need for stored food since, formed underground and dependent on subterranean organs for nourishment, it shoots up with rapid cell expansion and after a brief period of reproductive activity dies away.

The irregularity in occurrence and form of the "leaf gap" suggesting that it is not a well established character of the plant, raises the question as to whether it is to be regarded as a comparatively recent acquisition in the genus or as an ancient feature that is gradually disappearing. That it may be a rather new development might be concluded from its exceptionally strong formation in the fertile stem of *E. arvense*, which, if Schaffner's (7) view be accepted, is a phylogenetically recent organ. The fertile stem also is provided

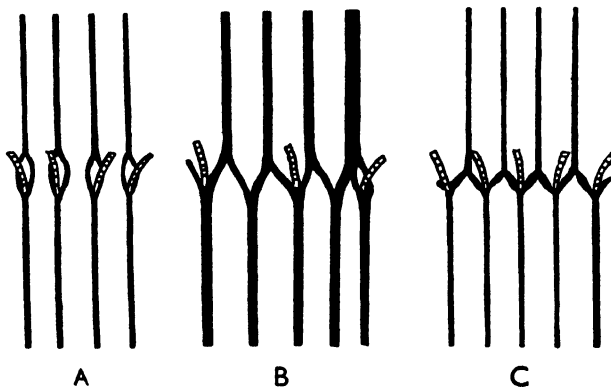


FIG. 2. (From Walton (8)). The relation between internodal bundles (solid) and leaf traces (hatched). A: *Asterocalamites*. B: *Calamites*. C: *Equisetum*.

with a large leaf sheath; this might be expected to involve the presence of a gap since the passage outward through the axial stele of a considerable mass of conducting tissue, such as the trace supplying a large leaf, should leave behind a gap in the main stele. On the other hand, a suggestion that the gap may be not recent, but long established, is found in the fact that, in an early stage of the development of the nodal region, the young stem bundle as figured for *E. arvense* by Queva (6) is cut into two similar halves by a wide parenchyma band, the result of the leaf trace having carried out the central part of the bundle. Moreover, a comparison of *Equisetum* with the

Devonian fossil *Asterocalamites*, to which *Equisetum* is doubtless related, gives further indication that the gap may not be recent. *Asterocalamites* had large leaves and if a gap accompanied these leaves and was not associated with leaf base storage, it would be an indication either of a true megaphyllous organization or of an enation type of leaf that had attained sufficient size for its trace to leave a gap in the vascular tissue of the stem. A diagram in a textbook by Walton (8, p. 80) showing the course of the vascular strands in *Asterocalamites* suggests that a leaf gap did occur in this genus (Fig. 2A). The arrangement of the cauline vascular tissue differs from that found in *Equisetum* (Fig. 2C) in that the longitudinal bundles did not fork at the nodes; the result was, therefore, that the leaves, not the branches, alternated with the parenchyma strands of the stem. If, in the course of evolution, there was a gradual shift from one condition to the other, it would not be difficult to imagine that a mass of parenchyma such as that figured by Walton as enclosed by the vascular loop above the departing leaf trace might have become reduced to the narrow parenchyma band that may occur to-day above the leaf trace of *Equisetum*. However, even the acceptance of such an homology does not decide the interpretation of the *Equisetum* "leaf gap" since no reference is made to the *Asterocalamites* "gap" by Walton in the text, and it cannot be determined from the diagram alone whether it is to be considered a true foliar gap.

That the absence of the "gap" from the cone in any way affects the question of a leaf gap in the plant depends on the consideration of the sporangiophore as a primarily foliar organ, an interpretation that is by no means universally accepted. To those who favour the view that the sporangiophore is of a foliar nature its absence would be an indication of the relatively recent appearance of the gap since without doubt the lines of evolution of stem and cone have been divergent, whether the cone is looked upon as a primitive region retaining ancestral features or as a specialized region developing characters distinct from those of the vegetative stem.

There remains the question of the bearing of these observations on Jeffrey's Lycopsid-Pteropsid classification. Since the classification was presented, there have been various proposals to transfer the Equisetales, a family comprising *Equisetum* and its fossil allies, to a separate group, the Sphenopsida (Articulatae). Two closely related fossil families were also to be placed in this group that was to rank equally with the Lycopsidea and the Pteropsida. In this connection, various lines of evidence have been brought forward, particularly by fossil botanists who emphasize the verticillate organization of the Sphenopsids. Still further evidence for the separation of the Equisetales from the Lycopsidea is offered by the presence, in at least the living genus of the family, of the peculiar type of "leaf gap" described above, no matter what interpretation is placed on this structure.

In conclusion it should be noted that before there can be a satisfactory understanding of the meaning of the "leaf gap" development in *Equisetum*,

a further detailed investigation of the nodal anatomy of the genus *Asterocalamites* must be made with a view to determining whether a true leaf gap occurred in this large-leaved primitive form.

Acknowledgments

This work was carried out in the Department of Botany, University of Toronto, under the direction of Professor R. B. Thomson, to whom the author wishes to express her thanks for his kind interest and help; she wishes also to thank several other members of the Department who have assisted her in various aspects of the work.

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AGRICULTURAL METEOROLOGY: MONTHLY SEQUENCE OF SUMMER PRECIPITATION AT WINNIPEG, SWIFT CURRENT, AND EDMONTON¹

By J. W. HOPKINS²

Abstract

An analysis of the monthly sequence of rainfall during the summer period April-September of the years 1890-1937, made by expressing each annual sequence as a fifth-degree polynomial function of time, indicates that despite marked annual variability the average monthly precipitation at all three stations exhibits a definite seasonal trend, with the maximum incidence of rain in June or July. At Winnipeg, the relative monthly distribution remains, on the average, essentially the same in both wet and dry years, but at Swift Current and Edmonton it is modified to some extent, the mid-season maximum being relatively more pronounced in seasons of above-average total precipitation. At Swift Current, both the total amount and one of the coefficients specifying the monthly distribution of precipitation show some oscillatory variation with time; and at Edmonton, there has been a slight progressive change such that a smaller proportion than formerly of the total precipitation now falls in the second half of the season (July-September). No consistent increase or decrease in rainfall over the 48-year period has been recorded at any of the three stations.

Introduction

In semi-arid regions, crop production may be markedly affected by the distribution as well as by the total amount of rainfall during the growing season. As has been pointed out by Barnes (1), it is a fortunate circumstance that in the Prairie Provinces of Canada this distribution is, on the average, favourable, the major part of the spring and summer precipitation in this region falling during the months of May, June, and July, when cereal plant growth is most rapid and most in need of moisture.

It is already well known (8) that the total amount of rain falling at any point in this region is subject to large annual fluctuations, partly of an irregular and at present unpredictable nature, but also showing some element of secular trend, owing to the alternation of periods that are, on the whole, of above- and below-average precipitation, although these are too inconstant as to both phase and amplitude to be reducible to any simple cycle of recurrence. The accompanying variations in the seasonal distribution of the total precipitation received have not, however, as yet been studied in any detail, although such

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² Statistician.

investigations as have been made in other countries suggest that these variations may be a feature of considerable significance. Thus Fisher (3), by the introduction of novel methods, was able to demonstrate from the records of rainfall at Rothamsted, England, for the period 1854-1918, a progressive change in the sequence of precipitation throughout the year, of which the main feature was an increase in December rain, with perhaps some relative reduction in spring and autumn. Likewise Cornish (2) applying the same procedure to the records of precipitation at Adelaide, South Australia, found that whilst there was no statistically significant change in the annual totals over the 95-year period, 1839-1933, there was a definite oscillation, with a period of approximately 23 years and an amplitude of 30 days, in the incidence and duration of the important winter rains. Succeeding portions of the present paper accordingly examine some statistics bearing upon this aspect of the climate of the Prairie Provinces.

Data and Method

The data used were the monthly totals of precipitation, expressed as inches of rain, for the period April to September inclusive, in each of the years 1890 to 1937, recorded by the Meteorological Service of Canada (9) at three representative stations. These were Winnipeg, Manitoba (lat. $49^{\circ} 53' N.$, long. $97^{\circ} 7' W.$, alt. 760 ft.); Swift Current, Saskatchewan ($50^{\circ} 20' N.$, $107^{\circ} 45' W.$, 2440 ft.); and Edmonton, Alberta ($53^{\circ} 33' N.$, $113^{\circ} 30' W.$, 2158 ft.).

For quantitative examination of the variations in seasonal distribution, the six monthly totals for each year at each station were first reduced to hundredths of an inch per day, in order to allow for differences in the number of days per month. Then the resulting series of six values was expressed, using Fisher and Yates's table of multipliers (5, Table XXIII), as a polynomial function of time of the fifth degree. The analytic advantage of this is that the individual monthly values are replaced by six polynomial coefficients a', b', \dots, f' , each of which provides a measure of a different component of the sequence as a whole; thus, b' is indicative of any linear increase or decrease in the amounts of rain recorded from the first to the sixth month, c' of the tendency of the sequence to a central maximum or minimum, d' of non-linear asymmetry, and so on. Furthermore, as the terms of different degree $\xi'_0, \xi'_1, \dots, \xi'_5$ are mutually orthogonal, the variation of the coefficient of each may be examined independently of that of the others.

Analysis of Rainfall Sequence

Tables I, II, and III list the distribution coefficients computed for each year and station.

Features of Average Sequence

All of the six coefficients $a' \dots f'$ in each table have a wide range of fluctuation, and, with the exception of a' , which is necessarily positive, exhibit numerous alternations in sign. However, the t ratios (4) shown at the foot

TABLE I
SEASONAL RAINFALL COEFFICIENTS, WINNIPEG

Year	a'	b'	c'	d'	e'	f'
1890	8.8	60.2	-43.7	-55.3	24.3	84.7
1891	8.1	37.6	-46.9	-14.9	7.7	-131.5
1892	7.4	1.3	-24.3	-107.7	-5.7	24.9
1893	8.7	-29.8	-84.2	-29.8	34.4	52.0
1894	5.6	-27.2	51.6	-3.2	26.0	-67.6
1895	6.6	-15.0	-59.1	58.5	-3.7	74.7
1896	11.2	-103.4	25.1	49.1	-0.3	-32.3
1897	6.3	-7.5	-86.2	-37.0	29.8	104.2
1898	7.9	22.4	-55.8	55.4	34.2	-161.0
1899	7.8	-15.3	-41.5	-24.3	-6.9	-83.7
1900	7.8	106.6	-13.9	-41.9	17.1	25.1
1901	11.6	20.9	-86.0	95.4	86.4	-250.2
1902	7.1	-24.2	-23.2	106.8	-3.8	-22.2
1903	7.3	19.7	-12.1	63.7	-30.7	131.9
1904	8.5	26.3	-99.9	12.3	39.1	45.3
1905	8.5	1.7	-102.9	71.7	18.9	21.9
1906	9.4	-28.5	-89.0	75.0	32.6	-75.0
1907	6.5	31.2	-59.3	-102.3	-5.7	28.5
1908	7.6	-7.6	-21.5	33.9	-8.5	-37.5
1909	7.4	24.7	-52.8	-124.8	-15.6	13.2
1910	6.2	20.5	16.8	31.0	-1.4	-56.8
1911	10.3	-39.9	-13.0	81.6	-33.4	84.0
1912	10.9	51.8	21.3	31.3	20.5	209.3
1913	6.7	50.3	-59.3	-63.7	-9.9	-106.7
1914	8.3	47.5	-73.0	-56.0	30.2	179.6
1915	6.4	50.3	42.9	85.3	40.5	-3.1
1916	7.7	23.3	-68.2	49.8	6.8	-37.2
1917	5.1	48.1	-40.5	-22.9	25.5	12.7
1918	6.4	5.5	-56.0	-41.0	5.6	-101.8
1919	10.0	42.9	-60.0	23.4	23.0	-54.0
1920	6.1	43.5	-5.1	81.5	8.5	-80.9
1921	8.2	42.9	9.1	-13.1	11.9	62.9
1922	8.0	37.6	-48.8	36.6	20.2	112.8
1923	5.5	-5.7	-45.9	22.3	7.9	97.7
1924	6.8	7.1	49.7	-52.9	31.5	40.9
1925	5.3	41.2	7.9	-16.3	-0.1	-98.3
1926	7.4	77.8	-21.3	27.3	10.3	-79.5
1927	8.5	-18.3	19.8	53.2	-37.2	7.4
1928	8.9	-8.3	-95.3	-16.3	-4.5	24.1
1929	5.1	-0.8	16.1	73.7	-3.5	27.7
1930	8.0	-9.8	-87.1	50.7	7.5	136.5
1931	6.7	50.7	-9.6	33.2	-3.6	77.2
1932	5.8	19.2	-28.2	3.2	23.2	-27.8
1933	8.2	14.6	-0.8	57.6	-57.0	52.2
1934	8.3	75.7	-3.6	30.2	18.6	-112.4
1935	8.4	29.3	-54.3	-30.7	-13.1	-123.5
1936	4.5	20.3	-19.1	44.3	22.5	-7.7
1937	7.9	-5.5	0.9	-9.5	8.3	14.3
Mean	7.6	16.8	-31.9	12.0	8.9	-0.1
Standard deviation	1.6	36.1	41.1	55.4	23.7	13.2
t	—	3.218**	5.374**	1.496	2.605*	0.008

* Exceeds 5% level of significance.

** Exceeds 1% level of significance.

TABLE II

SEASONAL RAINFALL COEFFICIENTS, SWIFT CURRENT

Year	<i>a'</i>	<i>b'</i>	<i>c'</i>	<i>d'</i>	<i>e'</i>	<i>f'</i>
1890	6.5	37.8	-12.1	36.3	1.5	-102.9
1891	9.7	9.9	-95.0	3.4	35.6	-151.6
1892	7.6	-71.0	-16.0	24.0	-1.8	-87.0
1893	3.8	33.3	-41.5	-74.7	0.1	62.1
1894	3.7	-28.1	-9.8	51.4	-12.6	7.4
1895	5.0	2.3	-73.5	45.3	24.5	32.1
1896	5.6	12.7	10.0	40.2	-33.0	-31.2
1897	6.1	69.3	-51.9	-50.7	40.3	165.9
1898	5.4	10.4	-55.4	-8.6	10.2	-1.0
1899	8.2	31.4	-101.7	-55.1	-19.5	-15.7
1900	6.5	40.4	-18.0	15.4	-16.2	34.4
1901	8.3	43.1	-48.0	89.6	45.0	33.4
1902	7.7	-33.9	-95.2	121.6	-15.4	-14.2
1903	7.9	7.5	-71.9	-15.5	-12.7	61.7
1904	5.3	23.8	-46.9	4.3	13.3	7.7
1905	7.4	-32.1	-77.2	80.4	19.0	65.4
1906	7.9	-25.8	-78.3	138.7	12.9	-204.1
1907	5.2	15.7	-26.8	-51.8	-23.0	-46.0
1908	3.5	-7.3	-39.8	18.2	9.4	-84.2
1909	9.3	-4.9	-142.4	35.6	30.6	-60.2
1910	4.8	13.0	-32.2	-28.0	1.4	-38.0
1911	6.0	8.3	-51.9	-11.7	1.1	-17.1
1912	6.0	8.4	-50.0	14.4	-17.0	12.6
1913	5.4	-2.1	-65.4	4.4	8.2	-56.0
1914	3.5	27.6	-0.4	42.6	22.6	-51.6
1915	6.2	-14.4	-62.1	106.1	-11.3	45.7
1916	7.1	18.6	-67.5	11.1	11.7	-59.7
1917	3.6	13.6	2.8	-4.8	14.0	-62.8
1918	3.4	.5	-14.1	-18.5	8.1	-30.7
1919	4.6	5.8	23.4	-72.2	-20.8	-40.0
1920	4.9	-2.0	-43.0	35.0	2.6	17.8
1921	7.0	51.6	11.9	76.1	13.3	37.9
1922	6.5	-32.9	-77.3	74.1	-7.9	-102.9
1923	7.5	-13.1	-143.6	17.4	53.6	-100.2
1924	5.8	5.7	-66.4	20.2	-17.2	-4.6
1925	6.0	14.9	31.5	-19.1	13.3	-8.5
1926	6.6	24.9	-63.1	-5.1	-24.1	-9.3
1927	10.1	10.0	-12.5	92.5	-45.9	142.7
1928	5.0	-15.3	-89.7	20.7	44.5	-71.1
1929	5.0	-10.2	-34.4	74.8	10.2	-25.0
1930	6.1	12.1	6.6	39.6	25.4	-129.6
1931	4.9	45.3	-14.1	18.3	5.3	-61.5
1932	8.1	21.4	-98.7	-126.1	17.5	56.5
1933	7.2	29.5	-19.9	-11.5	-33.1	-110.3
1934	5.0	18.5	-52.5	74.5	39.9	-111.1
1935	6.3	-18.9	-76.2	-37.4	22.0	-28.0
1936	4.0	.6	-31.5	23.1	-.7	-71.7
1937	2.4	12.7	-6.9	7.7	-3.9	19.9
Mean	6.0	7.7	-45.6	18.0	5.0	-24.7
Standard deviation	1.7	25.5	39.6	52.7	23.3	70.5
<i>t</i>	—	2.090*	7.973**	2.371*	1.490	2.425*

* Exceeds 5% level of significance.

** Exceeds 1% level of significance.

TABLE III
SEASONAL RAINFALL COEFFICIENTS, EDMONTON

Year	a'	b'	c'	d'	e'	f'
1890	10.0	71.2	-89.2	-13.8	5.4	37.2
1891	7.6	34.5	-98.8	-65.0	38.2	111.2
1892	5.8	17.9	-61.2	-46.6	15.2	-84.2
1893	6.9	-15.7	-82.7	53.3	19.3	73.3
1894	6.4	13.0	-49.1	78.5	-8.7	-52.7
1895	5.8	16.4	-7.0	-11.6	12.6	-37.6
1896	5.5	-17.1	-51.5	-10.1	-3.3	-24.7
1897	6.2	17.2	-91.1	-9.3	44.5	-17.7
1898	3.8	19.5	-49.5	12.5	25.1	-35.3
1899	9.0	22.7	-55.7	-99.3	-41.1	-91.5
1900	10.1	-5.6	-56.0	-53.6	-2.8	-28.0
1901	11.5	47.2	-121.0	-39.8	79.4	285.8
1902	10.9	7.0	-22.6	171.0	-37.0	163.8
1903	8.8	27.3	-83.2	-51.2	8.0	-95.2
1904	6.0	16.3	-78.3	16.3	29.7	-67.3
1905	7.3	32.3	-82.0	43.8	28.4	-18.6
1906	6.1	9.0	-59.8	56.0	29.2	-37.4
1907	7.3	26.7	-81.2	-56.8	-21.0	-57.8
1908	7.6	-6.3	-83.9	73.7	15.7	-87.5
1909	6.2	-4.0	-37.7	40.5	4.5	78.3
1910	5.2	8.9	-71.3	-36.1	-5.3	-46.1
1911	10.1	56.7	-105.8	-55.8	8.8	25.2
1912	9.3	15.6	-81.3	-76.9	-5.9	18.1
1913	8.6	42.3	-87.3	-96.7	5.5	-46.7
1914	9.3	-9.0	-154.5	57.5	39.1	-190.1
1915	9.9	47.9	-77.8	7.4	32.6	-69.8
1916	7.4	17.1	-59.7	-54.9	-6.9	-11.7
1917	6.8	51.5	-11.4	-31.0	9.2	20.8
1918	6.5	1.6	-70.1	-29.9	-1.7	-46.3
1919	5.1	-1.9	-18.6	34.6	-8.2	81.8
1920	7.4	-2.2	-66.7	49.3	9.9	-66.1
1921	6.1	20.7	-70.0	4.2	22.4	13.8
1922	5.3	9.7	-15.8	-72.8	-20.8	-17.8
1923	7.0	13.4	-91.9	-1.1	9.9	-44.5
1924	6.7	26.0	-71.1	-53.5	5.1	45.1
1925	6.0	-21.9	-15.3	-57.9	1.7	-57.3
1926	8.3	19.3	-76.8	-2.2	-41.4	-54.8
1927	7.4	28.2	-48.6	50.2	32.0	60.8
1928	7.6	24.5	-53.8	-5.0	19.2	98.0
1929	5.7	9.0	-41.9	-77.5	14.3	40.3
1930	4.7	-10.8	-53.1	12.7	1.7	-20.5
1931	9.1	26.6	-110.0	-4	7.4	-135.8
1932	5.6	-31.6	-34.3	-14.1	23.1	50.1
1933	6.6	-2	-74.9	14.3	18.9	16.9
1934	8.2	-8.9	-53.2	49.6	14.0	-23.2
1935	7.7	-40.1	-35.6	8.4	-8.8	-80.4
1936	5.9	-14.4	-35.6	33.6	-17.2	24.0
1937	8.2	43.3	-85.7	-70.7	36.9	244.7
Mean	7.3	13.6	-64.9	-6.8	9.1	-3.3
Standard deviation	1.7	23.1	29.9	49.8	24.4	87.2
t	—	4.067**	15.029**	0.947	2.585*	0.260

* Exceeds 5% level of significance.

** Exceeds 1% level of significance.

of each column of b' . . . f' , suggest that the mean values of b' , c' , and e' for Winnipeg and Edmonton, and of b' , c' , d' , and f' for Swift Current do differ significantly from zero. It is true that this test of significance can only be regarded as approximate when applied to distributions differing appreciably from normality; but the indications are that, in spite of very pronounced annual fluctuations, the average incidence of rain during the spring and summer months does follow a definite sequence at all three stations.

The second-degree coefficient, c' , reflects the most pronounced feature of the average sequences, namely, a maximum incidence of rain in the middle

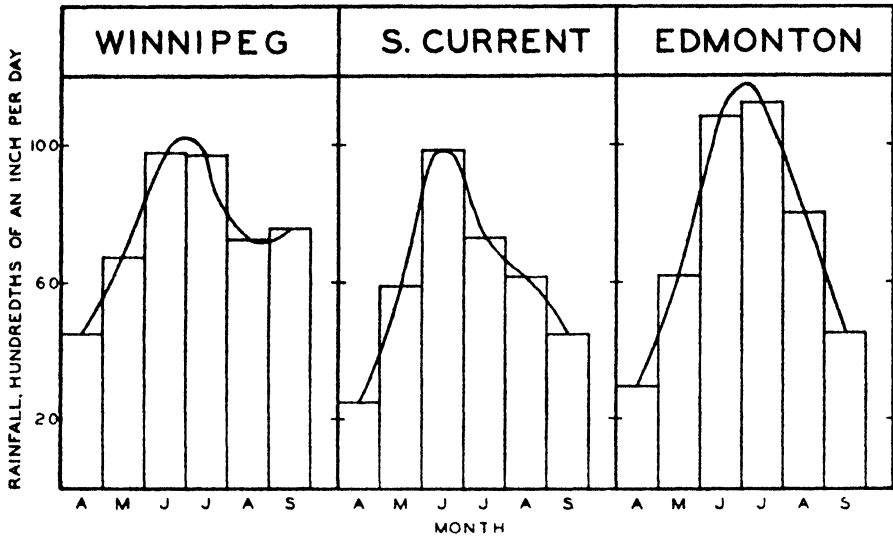


FIG. 1. Average monthly sequence of summer rainfall at Winnipeg, Swift Current, and Edmonton, 1890-1937.

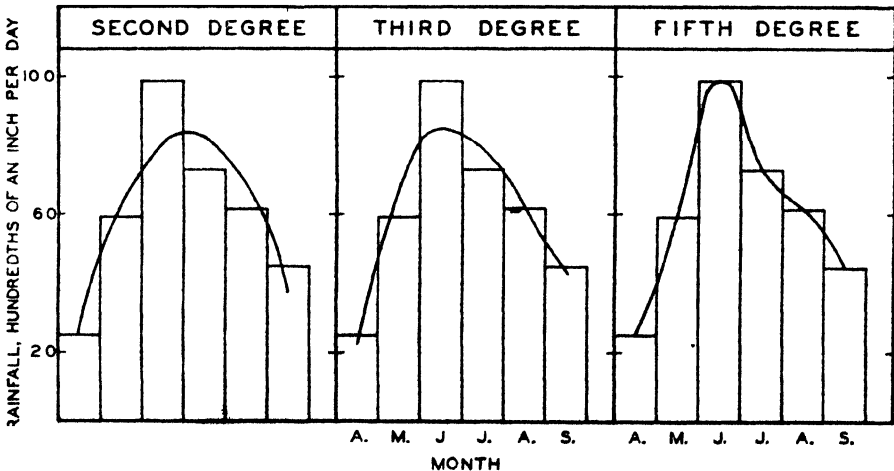


FIG. 2. Successive approximations to average monthly sequence of summer rainfall 1890-1937 at Swift Current by polynomials of second, third, and fifth degree.

part of the season, i.e., in June or July. This is illustrated in Fig. 1, which shows the 48-year monthly average for each location. The significance of the higher order terms d' and f' at Swift Current, and e' at Winnipeg and Edmonton, is occasioned by various inequalities in the rate of increase or decrease in precipitation in successive months. These are identifiable in Fig. 1, as well as more specifically in Fig. 2, which illustrates successive approximations to the average sequence at Swift Current by polynomials of the second, third, and fifth orders.

As might be inferred from the significance of the polynomial coefficients, the average sequence recorded at Swift Current differs from that at either Winnipeg or Edmonton, most notably in the marked decrease in rainfall in July and August. The maintenance of precipitation in September at Winnipeg is also worthy of remark.

Variance and Covariance of Annual Coefficients

As was noted in the preceding section, all of the rainfall coefficients, both of amount (a') and of distribution (b' . . . f') show large annual variations; the standard deviation of a' , for example, (comprising, in a normal distribution, about one-quarter of the main range) is itself 21% of the mean at Winnipeg, 29% at Swift Current, and 24% at Edmonton. Normality of the frequency distribution of the annual values of a' . . . f' for each station was tested by calculation of the statistics g_1 and g_2 (4). In this way it was found that none of the distributions of the Winnipeg coefficients deviated from normality to an extent demonstrable in a series of 48. However, e' and f' for Edmonton, and a' , b' and f' for Swift Current, all gave significantly positive values of g_2 , indicative of a clustering of the majority of these coefficients in the central region of the range of variation, with, however, a small number of rather markedly aberrant individuals; whilst f' for both Edmonton and Swift Current was also characterized by a positive g_1 , i.e., positive skewness.

Table IV shows the coefficients of correlation of the annual values of a' (proportional to the total seasonal rainfall) with those of the distribution coefficients b' . . . f' for each station. The correlation coefficients for Winnipeg are all statistically insignificant, from which it may be deduced that, on the average, the main features of the seasonal trend in precipitation were essentially similar in both wet and dry years at this location. This is seen to be the case from Fig. 3. In view of the deviation from normality of the frequency distribution of certain of the rainfall coefficients for Swift Current and Edmonton, "normal" tests of the significance of correlation coefficients involving these quantities cannot be exact. The indications are, however, that at Swift Current c' tended to become increasingly negative with increasing a' . This is also the case at Edmonton, but here there is in addition a moderate degree of positive association between b' and a' . At these two stations, therefore, the characteristics of the rainfall sequence must be to some extent modified in seasons of above- and below-average total precipitation. The nature of this modification is illustrated in Fig. 3. At

TABLE IV

COEFFICIENTS OF CORRELATION BETWEEN RAINFALL COEFFICIENTS OF AMOUNT (a') AND OF DISTRIBUTION (b' . . . f')

Rainfall coefficients	Coefficients of correlation		
	Winnipeg	Swift Current	Edmonton
a' and b'	-0 18	-0 05	0.43**
a' and c'	- 23	- 48**	- .49**
a' and d'	18	.19	.03
a' and e'	12	.00	-.05
a' and f'	- 00	03	.17

** Exceeds 1% level of significance ($r = \pm 0.37$).

Swift Current, where c' was correlated with a' , the concentration of rain in the middle of the season, with a maximum in June, is much more pronounced in the seasons of above-average totals. Both b' and c' were correlated with a' at Edmonton, and here it is seen from Fig. 3 that the excess of rain in the above-average seasons is most pronounced in July, and is also notably greater in September than in April. A qualitative, as well as a quantitative difference between wet and dry seasons at these points is thus indicated, which may be expected to affect not only the yield (7) but also the chemical composition of crops (6).

Secular Trend

In order to determine whether the variations in precipitation described above exhibited any degree of orderly sequence in time, or seemed to be

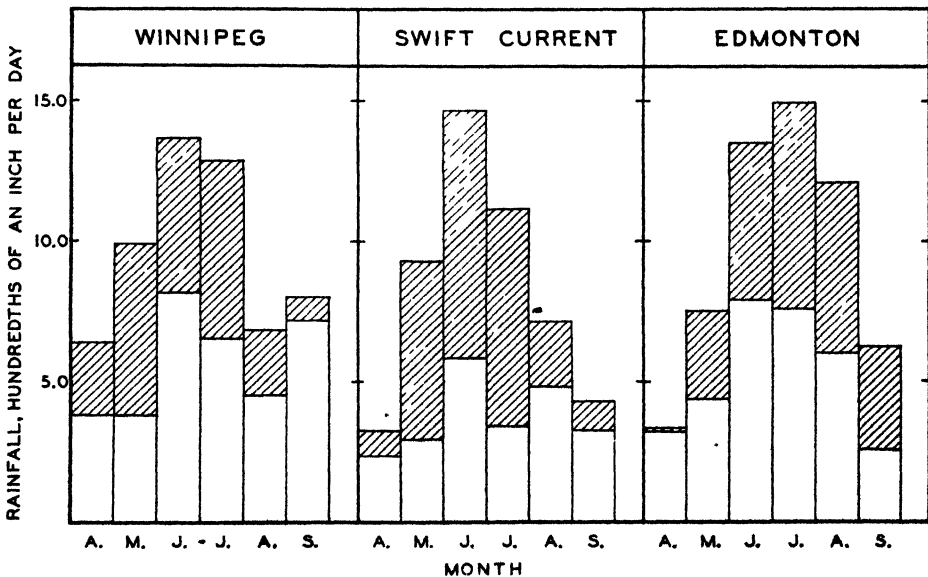


FIG. 3. Average monthly sequence of rainfall during the 12 driest (unshaded columns) and 12 wettest summers (shaded columns), 1890-1937.

essentially fortuitous or random in their incidence, the 48 annual values of a' . . . f' for each station were themselves subjected to a regression analysis in which a polynomial function of time of the fifth degree was fitted to each series, again by the use of Fisher and Yates's tables (5). Significance of the regression coefficients thus obtained was tested by the analysis of variance procedure (4), with the results summarized in Table V.

These indicate that none of the six coefficients for Winnipeg exhibits any demonstrable secular trend, but that both a' and f' for Swift Current do show some element of systematic variation with time. This is, however, of an

TABLE V
ANALYSIS OF VARIANCE OF RAINFALL COEFFICIENTS

Source of variance	Degrees of freedom	Mean square					
		a'	b'	c'	d'	e'	f
Winnipeg:							
Progressive increase or decrease	1	7.11	3147	3570	3031	1289	828
Non-linear trends	4	0.88	1983	956	4290	333	3563
Residual	42	2.58	1199	1713	2957	567	9047
Swift Current:							
Progressive increase or decrease	1	4.32	6	671	121	15	3548
Non-linear trends	4	6.89*	179	1355	647	82	13514*
Residual	42	2.61	708	1612	3048	547	4194
Edmonton:							
Progressive increase or decrease	1	2.02	1987*	1128	61	202	768
Non-linear trends	4	4.24	754	1257	1281	98	6746
Residual	42	2.99	478	854	3127	557	7841

* Exceeds mean square residual, 5% level of significance.

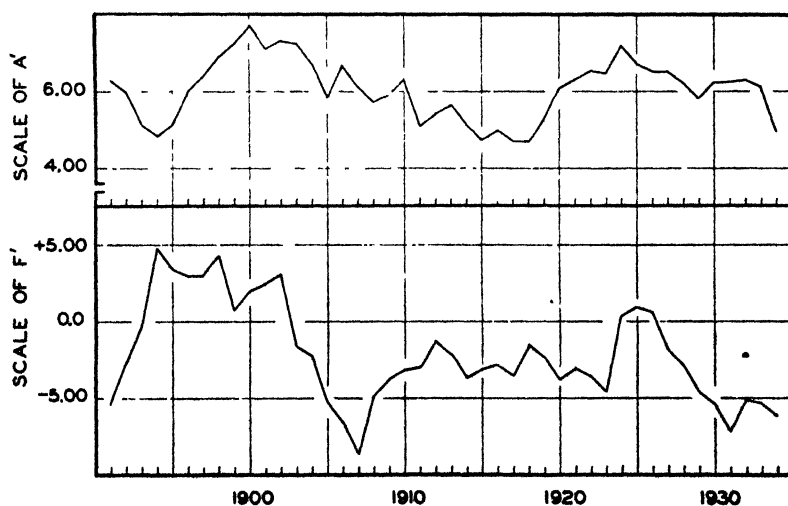


FIG. 4. Five-year moving averages of the rainfall coefficients a' and f' for Swift Current, 1890-1937.

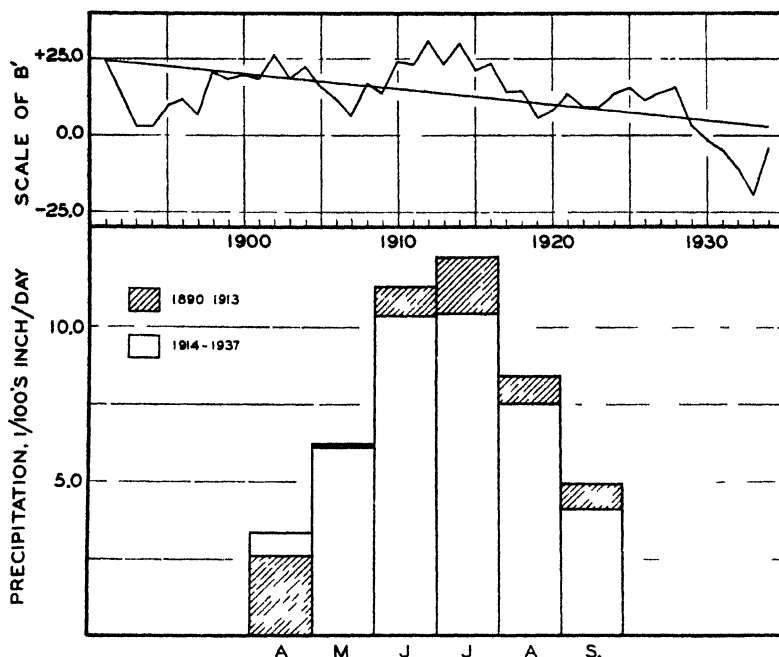


FIG. 5 Upper portion Five-year moving average and linear trend of the rainfall coefficient b' for Edmonton, 1890-1937. Lower portion: Average monthly sequence of rainfall at Edmonton, 1890-1913 and 1914-1937

oscillatory nature, neither showing any persistent tendency to increase or decrease over the 48 years as a whole. On the other hand, b' for Edmonton has on the average tended to decrease slightly during the period under review (Fig. 5), indicating some diminution in the proportion of the total precipitation occurring in the latter half of the season (July to September) relative to that falling in the months April to June. The lower portion of Fig. 5 illustrates this effect, which is the only progressive change in the seasonal distribution of precipitation demonstrable. It is noteworthy that there is no indication of any consistent increase or decrease in the total amount of precipitation recorded at any of the three stations.

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A SOLUTION FOR STAINING DIFFERENTIALLY THE SPORES AND VEGETATIVE CELLS OF MICRO-ORGANISMS¹

BY P. H. H. GRAY²

Abstract

A solution for staining differentially the spores and vegetative cytoplasm of bacteria, yeasts, and certain fungi has been developed. The solution is a mixture of two phenyl methane dyes, malachite green and basic fuchsin; it can be used as a concentrated aqueous solution or as a dilute saline solution. It is not necessary to use heat, though heating is recommended for staining "ripe" spores of bacteria. Decolorization and counterstaining are not required. Bacterial and yeast spores are stained blue or greenish-blue, vegetative cytoplasm light violet or pink. Young bacterial cells stain a deep violet, older cells light violet; the solution can thus be used as a general stain. The acid-fast organism *Mycobacterium berolinensis* was stained greenish-blue with the granules violet. The saline solution is recommended as a differential stain with young colonies of *Aspergillus* and *Penicillium*; the terminal growing tips and young branches of hyphae are stained blue, older hyphae light violet, spores and conidiophores blue.

Introduction

The methods that have been developed for staining the endospores of bacteria, the ascospores of yeasts, and the cells of "acid-fast" bacteria, require the application of heat to the stain with subsequent decolorization by acid, acid alcohol, or sodium sulphite, followed by a counterstain. The development of a method for staining the "vegetative" cells and the spores differentially by means of a single staining solution, in one operation, may prove to be a useful contribution to laboratory methods.

The Staining Solutions

Trials were made with aqueous solutions of malachite green containing different proportions of other phenyl methane dyes of contrasting colour. A mixture of malachite green and crystal violet stained spores greenish-blue and vegetative cytoplasm violet in films of *Bacillus mesentericus*, *B. megatherium*, and *B. subtilis*. The young spores were somewhat masked by an excess of violet colour, but the excess could be removed by washing for a few seconds with 95% ethyl alcohol, or by spreading a thin film of nigrosin over the stained cells. It was found that better results could be obtained by mixing basic fuchsin with the malachite green solution; with this mixture there was a more clear-cut differentiation. The proportions of these two dyes finally adopted were such that subsequent treatment with alcohol or nigrosin could be omitted, though, as stated below, the nigrosin may greatly improve the stain of some films. Two forms of the solution have been developed: an aqueous, concentrated solution and a saline dilution prepared from that.

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Solution A. The Aqueous, Concentrated Solution

The solution is composed of the following amounts of the dyes in distilled water:

Malachite green	0.50%
Fuchsin, basic	0.05%

It has been found convenient to prepare separate solutions of the two dyes, in double strength; solution may be hastened by placing the containers in a water bath at 56° C; equal volumes of these solutions are mixed to obtain the staining solution with the dyes in the proportions stated. The staining solution appears to be stable. It should be noted that as there may be differences in dye content in dyes of different manufacture, or in different batches from a factory, a slight modification in quantities may be necessary. The malachite green used in this laboratory was supplied by The British Drug Houses, Limited, Toronto; the basic fuchsin by Coleman and Bell.

Solution B. The Saline, Diluted Solution

This is prepared by diluting two parts of solution A with eight parts of a 0.8% sodium chloride solution. This mode of dilution was adopted since it was found that dilution of the aqueous, concentrated solution with water, or 10% glycerine solution, rapidly altered the cellular contents of the fungi used.

Methods of Staining and Organisms Used

Aerobic Spore-forming Bacteria

The method of staining that has proved consistently to give good differentiation with *B. megatherium*, *B. mesentericus*, and *B. subtilis*, is simple: the solution is allowed to act on fixed films for 3 or 4 min. at room temperature; the excess of stain is then washed off in running water, the film dried, and examined under the oil immersion objective. Spores in the early stage of development stain blue, in older stages, greenish-blue; ripe spores within the cell are tinged greenish-blue; free spores are unstained; the cytoplasm of vegetative cells stains violet in young cells, light violet in older cells, in some species pink rather than light violet.

Another simple method is to mix the cells in a loopful of the solution, on a slide, and allow it to dry. Fixing does not appear to be necessary. The excess of dry stain is washed off. The differentiation by this method is not so clear-cut as it is by the method described above.

By staining over steam, or by heating in a flame until steam rises, a sharper differentiation is obtained. The method adopted for heating has been to place the slide, with the stain on the film, on a 100 ml. beaker containing a little water; as soon as the water boils the flame is removed and the heating continued for 1 min., though less may be all that is required; the slide is then cooled, washed, and dried. By this method (with solution A) the young spores may be masked by excess of violet; this may be removed by washing for a few seconds with 95% ethyl alcohol, or by covering the film with a thin layer of a sterile 2% solution of nigrosin. A loopful of the nigrosin is placed

near the film after it has been stained, washed, and dried; by means of a strip of unsized (letterhead) paper, measuring one-half inch wide by one and one-half inches long, it is drawn, with firm pressure and rapid movement over the film on which it thus dries quickly. The nigrosin (which is not recommended for films that have been stained with the saline solution) is useful to demonstrate "capsules" in young cultures in which spores have begun to develop; these have been found in *B. megatherium**. Most of the violet colour is removed by the nigrosin but the spores are left stained.

"Acid-fast" Bacteria

Films were prepared from young cultures of "acid-fast" bacteria grown on slants of glycerol agar. Both solutions stained the cells of *Mycobacterium berolinensis* (both *r* and *s* forms) greenish-blue with the granules violet.

Smears of sputum were stained and the excess of violet removed by washing in 95% ethyl alcohol; organisms that resembled *Mycobacterium tuberculosis* were seen, stained greenish-blue in the light violet background. The film was then cleansed of oil and restained by the Ziehl-Neelsen method; the same cells were identified by their position on the slide, and found to be "acid-fast" bacteria. Neither solution (*A* or *B*) gives as clear-cut a differentiation as that given by the Ziehl-Neelsen method; moreover, a number of densely stained, greenish-blue short-rod and coccoid cells were observed, that were not "acid-fast" when stained by that method.

Other Bacteria

As indicated above, solution *A* is a good general stain, differentiating young from older cells. In it, cells of *Lactobacillus acidophilus*, after growth for 24 or 48 hr. in milk, stained light violet with granules a deep violet. *L. bulgaricus* also showed variability in cytoplasmic staining.

Yeasts

Two yeasts, *Saccharomyces cerevisiae* and a *Saccharomyces* obtained from fermenting apple juice, were found to form spores rapidly if grown on slopes of Bacto tryptone glucose-extract agar at 35° C.; spores began to develop in 48 hr., and after three weeks about 80% of the cells of the latter organism had formed ascospores.

In staining, the spores and vegetative cells are adequately differentiated by mixing a loopful of solution *A* with the yeast and placing a cover glass on the suspension. Many cells are stained a deep violet; the spores are greenish-blue; but they may not all stain simultaneously. Some cells do not stain. It has been observed that by this method, only one of the two or three spores in a large number of asci were stained.

The spores are well differentiated from the other cells by mixing the cells in the staining solution *A*, and allowing the film to dry, as described above. The fixed film, heated, gives better differentiation, with either solution.

* It has been found that broth is a better medium than water for preparing suspensions of this organism, which tends to "clump" in water.

After staining with solution *A*, it is necessary to use nigrosin in order to delineate the cell walls.

Aspergillus and Penicillium

The saline, diluted solution (*B*), was developed especially for use with "colonies" of these organisms grown on the surface of agar in plates. A clear medium is required; on Bacto potato dextrose agar, colonies were in suitable stages of development after incubation for 40 hr. at 35° C. *Aspergillus* is the better organism for demonstration since *Penicillium* forms a rather compact growth.

In staining, a drop of the solution is placed on a cover glass; this is inverted over the colony and pressure gently applied. The conidiophores and young spores stain greenish-blue, the terminal growing points of hyphae the same colour; older portions of hyphae become light violet, with young branches the same colour as the terminal portions. Ripe spores of *Aspergillus oryzae* remain brown with a tinge of green in the contents.

EFFECTS OF POTASSIUM ACID PHOSPHATE, CANE SUGAR, ETHYL MERCURIC BROMIDE, AND INDOLYLACETIC ACID IN A TALC CARRIER ON THE ROOTING OF STEM CUTTINGS¹

BY N. H. GRACE²

Abstract

Greenwood cuttings of *Deutzia Lemoinei*, *Symphoricarpos albus*, and *Weigela rosea* and dormant cuttings of *Lonicera tatarica* were treated with a series of 32 talc dusts containing potassium acid phosphate at concentrations of 0, 0.1, 1.0, and 10%, in combination with 0 and 10% cane sugar, 0 and 50 p.p.m. ethyl mercuric bromide, and 0 and 1000 p.p.m. indolylacetic acid. The lower concentrations of phosphate tended to increase rooting and reduce mortality of two of the species of greenwood cuttings whereas the 10% concentration was ineffective or injurious. However, this concentration was favourable to the rooting of dormant cuttings. Indolylacetic acid treatment increased the number of rooted cuttings and the number and length of roots. Beneficial effects were indicated for organic mercury and cane sugar treatments. However, these were attributed largely to the combinations with phosphate and indolylacetic acid. The results indicate that the effectiveness of dusts containing indolylacetic acid in the treatment of plant stem cuttings may be increased by the addition of nutrient and disinfectant chemicals.

In several communications have been reported the results of experiments in which plant cuttings were treated with talc dusts containing cane sugar, an organic mercurial disinfectant, and indolylacetic acid (3, 4, 8). Both cane sugar and the organic mercurial disinfectant produced beneficial effects under certain conditions. The foregoing work has been followed up by experiments in which inorganic nutrients have been added to the dust mixture (7). This communication describes experiments in which potassium acid phosphate was added to dusts containing cane sugar, ethyl mercuric bromide, and indolylacetic acid.

Experimental

The effects of potassium acid phosphate, cane sugar, ethyl mercuric bromide,* and indolylacetic acid were investigated by an experiment of factorial design. The series of dust mixtures comprised potassium acid phosphate at four concentrations, namely, 0, 0.1, 1, and 10%, in combination with cane sugar at 0 and 10%, ethyl mercuric bromide at 0 and 50 p.p.m., and indolylacetic acid at 0 and 1000 p.p.m. (parts of chemical to a million parts of the mixture with talc by weight). The complete series of possible dosage combinations of the four chemicals required 32 different talc dusts prepared by a grinding-mix operation from master dusts (7).

The experimental design involved three completely randomized replicates of the 32 treatments with 10 cuttings per treatment group. Four plants were

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* The ethyl mercuric bromide used in these experiments was prepared by a method developed in the Division of Chemistry, National Research Laboratories, Ottawa, by Dr. A. Cambron. This procedure yielded a product consisting of 80% ethyl mercuric bromide and 20% ethyl mercuric chloride.

TABLE I

ANALYSIS OF VARIANCE OF PLANT STEM CUTTINGS TREATED WITH TALC DUSTS CONTAINING POTASSIUM ACID PHOSPHATE, CANE SUGAR, ETHYL MERCURIC BROMIDE, AND INDOLYLACETIC ACID

Source of variance	Degrees of freedom	Mean square										
		<i>H. egelsii</i>		<i>Deutsia</i>		<i>Symphoricarpos</i>			<i>Lonicera</i>			
		Number of cuttings dead	Number of cuttings rooted and callused	Number of cuttings rooted	Weight of roots	Number of cuttings rooted	Number of roots per cutting rooted	Length of roots per cutting rooted	Mean root length	Number of cuttings rooted	Number of cuttings with new growth	Weight of new growth per cutting with new growth ($\times 10^3$)
Replicates	2	4923**	3950**	2018**	0 155	97 2	0 66	312	17 35	856 7**	294 7	9 833
Average treatment effects												
Organic mercury	1	1470*	446	1004*	0 108	582 1	0 51	233	18 64	51 3	7 9	1 584
Cane sugar	1	12	8	126	0 002	26 9	3 35	97	7 10	776 3*	1299 5**	15 251
Indolylacetic acid	1	807	2615**	5204**	1 741**	4913 5***	90 87***	9789***	0 47	8158 6**	306 7	0 001
Phosphate	3	1303**	1041**	604*	0 122	907 5**	5 94**	623	5 10	424 0*	309 0	10 504
Interactions												
Organic mercury \times cane sugar	1	28	23	14	0 110	431 8	0 04	375	10 60	270 0	8 4	35 651
Organic mercury \times indolylacetic acid	1	709	132	1	0 001	17 0	0 77	304	0 06	82 5	0 5	21 902
Organic mercury \times phosphate	3	289	285	562**	0 085	388 2	1 09	237	8 16	199 7	197 5	33 729*
Cane sugar \times indolylacetic acid	1	410	99	1386**	0 397	169 6	1 53	194	49 45*	822 5*	15 7	0 459
Cane sugar \times phosphate	3	198	281	232	0 051	214 4	0 51	198	5 19	184 7	30 5	5 929
Indolylacetic acid \times phosphate	3	133	44	153	0 066	259 0	1 59	541	18 49	235 9	41 1	1 612
Organic mercury \times cane sugar \times indolylacetic acid	1	93	485	65	0 070	34 1	0 01	68	6 35	87 8	252 2	26 334
Organic mercury \times cane sugar \times phosphate	3	315	523	215	0 234	78 7	0 73	114	5 43	75 1	190 0	22 584
Organic mercury \times indolylacetic acid \times phosphate	3	180	83	173	0 025	77 8	1 07	192	2 44	72 0	132 4	4 612
Cane sugar \times indolylacetic acid \times phosphate	3	106	308	74	0 032	140 3	0 63	46	4 88	261 1	62 0	33 337*
Organic mercury \times cane sugar \times indolylacetic acid \times phosphate	3	131	184	530*	0 331*	395 9	1 08	169	1 66	80 1	113 7	4 368
Error	62	292	193	172	0 102	156 1	1 09	276	11 67	122 2	118 4	10 581

*Exceeds mean square error, 5% level of significance.

**Exceeds mean square error, 1% level of significance.

***Exceeds mean square error, 0 1% level of significance.

used in the investigation, which thus required 960 cuttings of each species. Prepared cuttings* of the four species were sprayed with water (11), dusted in groups of 10, and planted immediately. Cuttings of *X Deutzia Lemoinei* Lemoine were planted August 16, 1939, and removed for examination September 15. Those of *Weigela rosea* Lindl. were planted August 16 and removed October 3. *Symphoricarpus albus* var. *laevigatus* Blake was planted September 1 and removed October 16. The greenwood cuttings of these three species were planted in brown sand in outside propagation frames in a garden. The frames were shaded with factory cotton screens. As heavy rains were encountered outside conditions were not particularly favourable. Dormant *Lonicera tatarica* L. cuttings were planted, on November 9, in brown sand in the greenhouse, under conditions that have been described (5), and removed December 20, 1939.

On removal, record was taken of the number of cuttings surviving, callused, and rooted, the number and length of roots and, for *Lonicera*, the number of dormant cuttings that produced new growth and the weight of new growth. The fresh root weight of *Deutzia* cuttings also was determined. The data were subjected to the analysis of variance procedure except in a few instances where poor rooting provided meagre data unsuited to statistical analysis. All counts of numbers of cuttings were subjected to the inverse sine transformation prior to analysis (1).

Results

Data for the analyses of variance of responses of the cuttings of the four species are given in Table I. Results for each of the four species has indicated some significant response to treatment with indolylacetic acid and potassium acid phosphate. Although effects from treatment with cane sugar and organic mercury are not so general, several are to be noted. There were also a number of interactions between the chemicals. Poor rooting of dormant *Lonicera* cuttings without indolylacetic acid treatment necessitated confining the analyses of variance of numbers and lengths of root and the mean root length to the 480 cuttings that received the phytohormone. As the only significant effect on the number of roots per rooted cutting was that attributable to cane sugar, the results of these analyses have not been tabulated. Data for some of the more important effects of treatments have been selected and are given in the following tables.

Weigela

In Table II are given data for the effects of potassium acid phosphate treatments. The data are averages for treatments with and without cane sugar, organic mercury, and indolylacetic acid. Both the 0.1 and 1% concentrations of phosphate resulted in an increase in the number of cuttings rooted and callused and also in reduced mortality, whereas the results with 10% level of phosphate did not differ from those in the controls.

* The prepared cuttings were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood.

TABLE II

AVERAGE EFFECTS OF POTASSIUM ACID PHOSPHATE IN TALC DUSTS ON THE RESPONSES OF *Weigela* CUTTINGS

	Kind of data	Concentration of potassium acid phosphate in dust, %				Necessary difference, 5% level
		0	0.1	1	10	
Number of cuttings rooted and callused	Transformed Per cent	16.29 14.6	27.56 27.5	26.38 24.2	14.97 13.3	8.03
Number of cuttings dead	Transformed Per cent	40.97 43.3	29.52 30.4	33.66 34.6	46.01 47.5	9.86

In the absence of organic mercury about 43% of the cuttings died, whereas in its presence mortality fell to 35%. Indolylacetic acid treatment, on the average, resulted in production of 27% of rooted or callused cuttings. In its absence the value was 13%.

Deutzia

The effects of potassium acid phosphate and organic mercury treatment on rooting are indicated in Table III, in which the data are averages for treatments with and without cane sugar and indolylacetic acid. Organic mercury increased rooting when combined with the 0.1 and 10% concentrations of phosphate. The high phosphate concentration was depressing in the absence, but not in the presence, of organic mercury.

Indolylacetic acid treatment, on the average, increased rooting from 20 to 40%. Whereas both indolylacetic acid and cane sugar increased rooting when applied separately, the effect was not additive when these chemicals were combined. At the 1% concentration of potassium acid phosphate,

TABLE III

INTERACTION EFFECTS OF POTASSIUM ACID PHOSPHATE AND ETHYL MERCURIC BROMIDE ON THE ROOTING OF *Deutzia* CUTTINGS

Concentration of organic mercury, p.p.m.	Kind of data	Concentration of potassium acid phosphate in dust, %				Mean of organic mercury treatment
		0	0.1	1	10	
0	Transformed Per cent	30.1 28.3	26.7 25.0	35.4 35.8	17.5 15.0	26.1
50	Transformed Per cent	34.1 34.2	42.8 46.7	29.4 28.3	29.2 26.7	34.0
Means of phosphate treatment	Transformed Per cent	32.1 31.3	34.7 35.8	32.4 32.1	23.3 20.8	

Necessary difference, 5% level: phosphate means, 7.57; interaction, 10.7.

organic mercury was more beneficial to rooting when in conjunction with both cane sugar and indolylacetic acid than with indolylacetic acid alone.

The average effect of indolylacetic acid treatment was to increase fresh root weight per group of 10 cuttings planted from 0.14 to 0.41 gm. Thus, whilst the number of rooted cuttings was approximately doubled, the weight of roots produced was increased about three times. The significant interaction between the four chemicals disclosed by the analysis of fresh root weights is related to the beneficial effect of organic mercury when in combination with indolylacetic acid, cane sugar, and the 1% concentration of phosphate.

TABLE IV

AVERAGE EFFECTS OF POTASSIUM ACID PHOSPHATE IN TALC DUSTS ON THE RESPONSES OF *Symphoricarpus* CUTTINGS

—	Kind of data	Concentration of potassium acid phosphate in dust, %				Necessary difference, 5% level
		0	0.1	1	10	
Number of cuttings rooted	Transformed Per cent	60.8	55.0	56.7	46.2	7.2
		72.5	64.2	67.9	51.7	
Number of roots per rooted cutting		3.9	3.7	3.4	2.8	0.6

Symphoricarpus

Effects of potassium acid phosphate on rooting and the number of roots are presented in Table IV, in which the data are averages for treatments with and without indolylacetic acid, cane sugar, and organic mercury. The 10% concentration of phosphate resulted in rooting percentage and number of roots per rooted cutting being below both the control groups and the groups receiving the two lower phosphate concentrations. Data for the average effects of indolylacetic acid treatment are given in Table V. The application of 1000 p.p.m. of indolylacetic acid in a talc carrier has resulted in marked increase in percentage of rooting and in both the number and lengths of root per rooted cutting.

TABLE V

AVERAGE EFFECTS OF INDOLYLACETIC ACID TREATMENT ON THE RESPONSES OF *Symphoricarpus* CUTTINGS

—	Indolylacetic acid in talc, p.p.m.	
	0	1000
Number of cuttings rooted, %	54.2	74.0
Number of roots per rooted cutting	2.5	4.4
Length of roots per cutting rooted, mm.	23.8	44.0

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	Kind of data	Concentration of potassium acid phosphate in dust, %				Necessary difference, 5% level
		0	0.1	1	10	
Number of cuttings rooted and callused	Transformed	16.29	27.56	26.38	14.97	8.03
	Per cent	14.6	27.5	24.2	13.3	
Number of cuttings dead	Transformed	40.97	29.52	33.66	46.01	9.86
	Per cent	43.3	30.4	34.6	47.5	

In the absence of organic mercury about 43% of the cuttings died, whereas in its presence mortality fell to 35%. Indolylacetic acid treatment, on the average, resulted in production of 27% of rooted or callused cuttings. In its absence the value was 13%.

Deutzia

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INTERACTION EFFECTS OF POTASSIUM ACID PHOSPHATE AND ETHYL MERCURIC BROMIDE ON THE ROOTING OF *Deutzia* CUTTINGS

Concentration of organic mercury, p.p.m.	Kind of data	Concentration of potassium acid phosphate in dust, %				Mean of organic mercury treatment
		0	0.1	1	10	
0	Transformed	30.1	26.7	35.4	17.5	26.1
	Per cent	28.3	25.0	35.8	15.0	
50	Transformed	34.1	42.8	29.4	29.2	34.0
	Per cent	34.2	46.7	28.3	26.7	
Means of phosphate treatment	Transformed	32.1	34.7	32.4	23.3	
	Per cent	31.3	35.8	32.1	20.8	

Necessary difference, 5% level: phosphate means, 7.57; interaction, 10.7.

organic mercury was more beneficial to rooting when in conjunction with both cane sugar and indolylacetic acid than with indolylacetic acid alone.

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		0	0.1	1	10	
Number of cuttings rooted	Transformed Per cent	60.8	55.0	56.7	46.2	7.2
		72.5	64.2	67.9	51.7	
Number of roots per rooted cutting		3.9	3.7	3.4	2.8	0.6

Symphoricarpus

Effects of potassium acid phosphate on rooting and the number of roots are presented in Table IV, in which the data are averages for treatments with and without indolylacetic acid, cane sugar, and organic mercury. The 10% concentration of phosphate resulted in rooting percentage and number of roots per rooted cutting being below both the control groups and the groups receiving the two lower phosphate concentrations. Data for the average effects of indolylacetic acid treatment are given in Table V. The application of 1000 p.p.m. of indolylacetic acid in a talc carrier has resulted in marked increase in percentage of rooting and in both the number and lengths of root per rooted cutting.

TABLE V

AVERAGE EFFECTS OF INDOLYLACETIC ACID TREATMENT ON THE RESPONSES OF *Symphoricarpus* CUTTINGS

—	Indolylacetic acid in talc, p.p.m.	
	0	1000
Number of cuttings rooted, %	54.2	74.0
Number of roots per rooted cutting	2.5	4.4
Length of roots per cutting rooted, mm.	23.8	44.0

The mean root length averaged 9.8 mm. over the entire experiment. Indolylacetic acid and cane sugar each had a slightly beneficial effect upon this attribute when applied separately, but in combination reduced the mean root length to 8.8 mm.

Lonicera

The effects of indolylacetic acid and cane sugar treatment on the number of cuttings rooted are described in Table VI, in which the data are averages for treatments with and without phosphate and organic mercury. Whereas indolylacetic acid markedly increased rooting, cane sugar had a depressing effect in the absence, but not in the presence of indolylacetic acid.

TABLE VI

INTERACTION EFFECTS OF INDOLYLACETIC ACID AND CANE SUGAR ON THE ROOTING OF DORMANT *Lonicera* CUTTINGS

Concentration of cane sugar in talc, %	Kind of data	Indolylacetic acid in talc, p.p.m.		Means
		0	1000	
0	Transformed	30.8	43.4	37.9
	Per cent	28.3	47.5	
10	Transformed	19.3	43.6	31.9
	Per cent	16.3	47.5	
Means	Per cent	22.3	47.5	

Necessary difference, 5% level, 6.38 (transformed data only).

Data for the average effects of potassium acid phosphate on the number of cuttings rooted are given in Table VII. An increase in rooting is suggested after treatment with 1% phosphate; after 10% phosphate the increase is significant. Stimulation of rooting of these dormant cuttings by the high level of phosphate is in marked contrast to the results with the other three species.

In the presence of cane sugar, 71% of the cuttings had new growth, as compared with 80% in its absence. A further partition of the variance shown

TABLE VII

AVERAGE EFFECTS OF POTASSIUM ACID PHOSPHATE IN TALC DUSTS ON THE ROOTING OF DORMANT *Lonicera* CUTTINGS

Kind of data	Concentration of potassium acid phosphate in dust, %				Necessary difference, 5% level
	0	0.1	1	10	
Transformed	31.8	30.9	34.1	40.2	6.4
Per cent	31.7	30.4	35.4	42.1	

in Table I demonstrated that phosphate at the 1 and 10% concentrations reduced the number of cuttings with new growth.

Data for the weight of new growth per cutting with new growth demonstrated that organic mercury was slightly beneficial in the absence of phosphate, slightly depressing in conjunction with both the 0.1 and 1% concentrations, and highly beneficial with the 10% concentration. The interaction of cane sugar, indolylacetic acid, and potassium phosphate was such that whereas indolylacetic acid increased the weight of new growth in the absence of cane sugar and phosphate, its combination with sugar or the several phosphate concentrations reduced weight of new growth. However, the combinations of indolylacetic acid with both sugar and phosphate were all beneficial, increasing the weight of new growth.

Cane sugar treatment, averaged over treatments with indolylacetic acid and with and without organic mercury and phosphate, increased the number of roots per rooted cutting from 4.1 to 5.4.

Discussion

The results are in general agreement with those of other experiments in which the responses of stem cuttings were affected by cane sugar, indolylacetic acid, and an organic mercurial disinfectant, and in which there were interactions between these chemicals (3, 4, 6, 7, 8). It has been shown that various nutrient solution treatments of cuttings have marked effects and nutrient chemicals other than sugars have been applied to cuttings with successful results (2, 6, 9-11). These results demonstrate that potassium acid phosphate has some significant effects on rooting, both when considered alone and in combination with the other chemical factors. Although the results do not warrant any general recommendation as to the most desirable concentration of phosphate, it would appear that greenwood cuttings are somewhat more sensitive to overdosage than those of dormant plants. Organic mercury treatment appeared to be particularly beneficial in conjunction with phosphate and both phosphate and cane sugar. The interaction of these chemicals with indolylacetic acid suggests that such combinations may be of some value in the propagation of cuttings of certain plant species.

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THE ACTION OF MICRO-ORGANISMS ON FAT

III. OXIDATION AND HYDROLYSIS OF TRIOLEIN BY PURE CULTURES OF BACTERIA¹

BY C. H. CASTELL² AND E. H. GARRARD³

Abstract

The hydrolytic activity of 40 pure cultures of bacteria on triolein, as indicated by increased titratable acidity, has been recorded. Schiff and Kreis tests have been made on similar samples of triolein acted upon by pure cultures of bacteria, and the results of these tests are compared with the "oxidase reaction" of the individual cultures.

The results indicate that most of the Gram-negative lipolytic organisms also oxidize the fat, and that there appears to be a definite relation between the "oxidase reaction" of a bacterial colony and the ability of the organisms to produce oxidative rancidity.

Introduction

Of all the non-nitrogenous constituents of food, from the standpoint of microbial activity, least is known about fat. A study of the processes in the fermentation industries and a study of the carbohydrate reactions, now used extensively in bacterial classification, has greatly increased the knowledge of the decomposition of starches, sugars, alcohols, and related substances. Not only is there a fairly detailed knowledge of the chain of reactions occurring in fermentations, but a general idea of the microbial species bringing them about is also known. The same cannot be said for the action of bacteria on fat. In Bergey's manual, rarely, if ever, is oxidative or hydrolytic activity on fats mentioned, and apart from a few general and widely spread references there appears to be no place in the literature where information regarding the action of specific bacteria on fats can be obtained.

In this paper is reported the action of some 40 species of bacteria on triolein⁴. The results are given in terms of hydrolysis, as indicated by the increase in titratable acidity in the oil, and oxidation, as shown by the Schiff and Kreis tests. For the purpose of comparison, the "oxidase" test devised by Gordon and McLeod (3) was also made on each of the cultures.

Apart from the general information obtained, these tests were planned so as to form a basis for comparing various spot tests and dye reactions frequently used for detecting the lipolytic activity of bacteria.

Triolein was used in preference to one of the more complex natural fats so as to lessen the number of factors involved. It was preferred to the more

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⁴ It was originally intended that nothing but pure triolein would be used throughout these and succeeding experiments. Unfortunately, owing to the war, the source of this pure fat was stopped, and efforts to obtain it elsewhere have met with failure. Most of the available supply of the pure triglyceride has been saved for later experiments, and in some of this preliminary work, approximately pure triolein, prepared by crystallizing and filtering olive oil, has been substituted.

available, saturated triglycerides, because of its capacity of being easily oxidized as well as hydrolysed.

Pure Cultures

Forty pure cultures of bacteria were used in the present series of experiments. The majority were obtained from the stock of pure cultures kept by Professor A. Davey, of this department. Some of the lipolytic organisms were kindly supplied by Dr. Hammer, of the Iowa State College, at Ames, Iowa. Most of the cultures were previously tested by their reactions in sugars and other differentiating media in order to verify their identity. The rather wide variety of species was used purposely in the hope of obtaining variations in lipolytic activity, as well as to determine the action of a large number of bacterial species on the fat.

Experimental Methods

Hydrolysis

In small, 50 ml. screw capped flasks, 20 ml. of triolein and 20 ml. of a 1% peptone solution were mixed and sterilized in the autoclave. These were inoculated with a loopful of actively growing organisms cultured on agar slants. The cultures were incubated at 25° C. (77° F.) and a second series, of 17 cultures was incubated at 37° C. (98° F.). Each day the flasks were shaken to thoroughly mix the aqueous suspension of bacteria with the fat. As the flasks were all tightly stoppered, and as the solution containing the bacteria was covered with a deep layer of oil, there was a tendency towards the production of anaerobic conditions. To determine whether this had any marked influence on hydrolysis of the fat, a third series of a few cultures was prepared, providing more aerobic conditions. Sections of wide glass tubing were suspended in wide-mouthed flasks so as to leave the peptone solution in the central portion exposed to the air. The cultures were incubated at 25° C. (77° F.).

After 11 and 17 days' incubation, samples of the oil were aseptically withdrawn from the flasks and titrated with N/10 sodium hydroxide in a neutral solution of equal parts of ether and alcohol, with phenolphthalein as the indicator. At least two titrations were made for each sample and, if the figures agreed, the result was recorded. A similar titration was made on a sample of the original oil. The figures in Table I represent the differences in values obtained for the inoculated samples and those of the original oil. The results are calculated in terms of oleic acid.

In some cultures duplicate tests were made; the results between similar cultures never varied more than 0.05%, the average variation being 0.04%. This was not the case when, as indicated in the table, different strains of the same culture were used.

Oxidation

The flasks for these tests were prepared in the same way as those for acid production. At the end of 17 days, a Kreis test and a Schiff test were made on each sample according to the methods given by Lea (6). As these tests

were numerous and only comparative results were required, the extent of the reactions was judged by comparing the colours and marking them from (+) to (++++) as the strength of the reaction increased. The results given are for samples incubated at 25° C. (77° F.)

The "oxidase" test was made by flooding agar plates of each culture with a weak solution of *p*-aminodimethylaniline monohydrochloride, as described in a previous paper (1).

Results and Discussion

The most significant results from these experiments are given in Table I. Of the 40 cultures tested for acid production at 25° C. (77° F.) only three produced more than 1% acidity; 11 produced more than 0.1%; 26 produced no acidity, and the remaining three less than 0.01%. Besides those listed in Table I, the following organisms produced no acid: *Bacillus subtilis*, *B. panis*, *B. graveolens*, *Salmonella enteritidis*, *S. aertrycke*, *Escherichia coli communior*, *Rhizobium trifolii*, and *Brucella abortus*. Several of these included two strains.

In the samples in which the oil was prevented from completely covering the surface of the peptone solution, there was a marked increase in the amount of fatty acid formed by some species. The most notable example was *Staphylococcus aureus*, which, under these conditions, produced 11.97% acid (10.33% more than in the regular samples); *Bacillus mesentericus* produced 0.29% acid (0.24% more) but *Alcaligenes viscosus*, on the other hand, produced slightly less acid under these conditions. It is probable that these results are owing mainly to differences in growing conditions for the bacteria.

It is interesting to note that an increase in temperature had a different effect on the amount of acid produced by different cultures. *Pseudomonas aeruginosa* (A29) and *S. aureus* show this difference well. Both have an optimum temperature of 37° C. (98° F.), yet when the acidities at 25° C. (77° F.) and 37° C. (98° F.) are compared, it is seen that there is a marked decrease in the acid production by *P. aeruginosa* at the higher temperature, whereas there is an equally marked increase by *S. aureus*. As shown by the Schiff and Kreis tests and the "oxidase" reaction, *P. aeruginosa* has a marked oxidizing action whereas *S. aureus* is among the least active in this respect. It is probable then that the differences noted above are owing to the increased oxidation by the enzymes of *P. aeruginosa* at the higher temperature.

In general it may be stated that, of the organisms tested, species of the *Pseudomonas*, *Phytomonas*, *Alcaligenes*, and *Achromobacter* genera and *S. aureus*, were the only organisms showing any distinct lipolytic action on triolein. There is as much variation between different strains of the same species as between different species, and temperature besides other cultural conditions has a marked effect on the amount of acid formed.

It should be realized that the lipolysis indicated by these tests refers only to triolein. Some of these organisms may produce butyrases capable of hydrolysing triglycerides of lower molecular weight and other simple fatty substances.

TABLE I

RESULTS OF THE KREIS AND SCHIFF TESTS, THE INCREASED TITRATABLE ACIDITY IN SAMPLES OF TRIOLEIN MIXED WITH A PEPTONE SOLUTION, INOCULATED WITH PURE CULTURES OF BACTERIA, AND INCUBATED FOR 17 DAYS AT 25° C., AND THE OXIDASE REACTION OF THESE SAME ORGANISMS AFTER 36 HR. ON STANDARD BEEF AGAR

Organism	Strain No.	Oxidase reaction	Kreis	Schiff	Acidity (% oleic acid)
<i>Pseudomonas</i>					
<i>aeruginosa</i>	A29	+++	+++	+++++	1.07 (0.18)*
<i>aeruginosa</i>	A29a	+++	+++++	+++++	0.73
<i>fluorescens</i>	3	+++	+++	+++++	0.51
<i>fluorescens</i>	M25	+++	+++++	+++++	1.30
<i>mucidolens</i>	6	+++	+++	+++	0.56
<i>fragi</i>	5	++	++	++	0.18
<i>Pythomonas</i>					
<i>campestris</i>	62	++	+++	+++	0.35
<i>Alcaligenes</i>					
<i>faecalis</i>	A4a	++	Omitted	+++	0
<i>lipolyticum</i>	8	+	Omitted	++	0.12
<i>lipolyticum</i>	1	++	Omitted	+++++	0.11
<i>Achromobacter</i>					
<i>putrefaciens</i>	2	+++	Omitted	+++++	0
<i>Erwinia</i>					
<i>caratovorora</i>	P3a	+	Omitted	+++	0
<i>Rhizobium</i>					
<i>leguminosarum</i>		++	Omitted	+++	0 (0)*
<i>Bacillus</i>					
<i>mesentericus</i>	18	+(?)	++	+++	0.05
<i>cereus</i>	19	—	+	+	0
<i>mycoides</i>	M9	—	+	+	0
<i>Proteus</i>					
<i>vulgaris</i>	A28	—	+	+++	0.05
<i>Alcaligenes</i>					
<i>viscosus</i>	M4	—	+++	++	0.70
<i>cloacene</i>	A2	—	+	+++	0
<i>aerogenes</i>	M3	—	+	++	0.09 (0.05)*
<i>Escherichia</i>					
<i>coli</i>	M15	—	+	++	0 (0)*
<i>Salmonella</i>					
<i>pullorum</i>	17	—	+	++	0 (0)*
<i>suipestifer</i>	A41	—	+	+++	0 (0.05)*
<i>Staphylococcus</i>					
<i>citreus</i>	A48	—?	++	+++	0
<i>aureus</i>	A47	—	+	+	1.64 (7.83)*
<i>Diplococcus</i>					
<i>capsulatus</i>	32	—	+	+++	0

NOTE: The number of (+) signs indicates the strength of the reaction, and (—) indicates no reaction. In the Kreis and Schiff tests the uninoculated controls were designated (+) and the others compared to these.

* The figures in parentheses represent the acidity produced by the same cultures when incubated at 37° C.

Oxidation reactions are much more complex and very much more difficult to measure than simple hydrolysis of the fat. The variations in the types of reaction and the multiplicity of products formed, produce results that cannot be estimated accurately by two simple tests. For that reason, it is realized that the results shown in Table I have a limited significance. In a

general way, however, it appears that organisms of the *Pseudomonas*, *Achromobacter*, *Alcaligenes*, and *Phytomonas* genera very actively catalyse oxidation of triolein and that most of the cocci and the bacilli tested are less active in this respect. Of these two groups, *Staphylococcus citreus* and *B. mesentericus* are apparently slight exceptions.

It is particularly interesting to note the close similarity between the results of the Kreis and Schiff tests and the "oxidase" reactions. There are a few exceptions to this. *Proteus vulgaris*, *Aerogenes cloaceae*, and one or two others that are oxidase-negative, gave a rather strong Schiff reaction, although the results of the Kreis test was almost the same in the controls.

These results agree substantially with those of other workers who have used various substrates and different methods for determining the lipolytic activity of bacteria. These have been reviewed by Jensen and Grettie (5) and Lea (6). The following authors also present confirmatory evidence; Collins (2) and Hussong (4) using a butterfat emulsion in agar as a substrate and Nile blue sulphate as an indicator, found *Pseudomonas fragi*, *P. fluorescens*, *P. mucidolens*, *Achromobacter lipolyticum*, and *A. conii* to be strongly lipolytic. Wells and Corper (8) by extracting lipase from bacteria and using ethyl butyrate, triacetin, and olive oil as enzyme substrates found *Staphylococcus* species and *P. aeruginosa* to be the most lipolytic of a number of organisms tested. Trussel and Weed (7) found 38 different strains of *Staphylococcus* to be lipolytic and that under aerobic conditions these organisms produced much more acid than when the air was excluded.

One chief difference noted was that *Escherichia coli* was frequently described as being lipolytic. In this connection it may be stated that the authors, using oil emulsion methods over a period of years have found considerable variation in the hydrolytic activity of what were considered to be cultures of *E. coli*, *Aerogenes aerogenes*, and *A. cloaceae* from different sources.

Finally, the results recorded here confirm the observations made by Jensen and Grettie (5), namely, that many of the most actively lipolytic bacteria were also able to bring about oxidation reactions in fat.

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THE ACTION OF MICRO-ORGANISMS ON FAT

IV. OBSERVATIONS ON THE CHANGES PRODUCED IN GLOBULES OF TRIOLEIN BY PURE CULTURES OF BACTERIA¹

BY C. H. CASTELL² AND E. H. GARRARD³

Abstract

A series of observations have been made on the action of 60 cultures of bacteria on globules of triolein in oil emulsion agar media. Lipolytic activity as indicated by the colour reactions of Nile blue sulphate and methylene blue and the blue soap formation with copper sulphate has been shown to coincide with that of the same organisms as measured by the titratable acidity they produced in larger samples of the oil. Other Eh indicators have been shown to colour the globules around lipolytic colonies.

Other changes in the colour and texture of the globules have been shown to coincide with the oxidative activity of the bacteria as measured by Kreis and Schiff tests on larger samples as well as by the oxidase reaction of the bacterial colony. Preliminary hydrolysis appears to hasten and intensify the oxidative reactions as indicated by the globules.

Other colour reactions in the globules and the formation of various types of crystals have been observed and their significance discussed.

Introduction

The most frequently used method of detecting the lipolytic action of bacteria is to observe the changes produced by the organisms in minute globules of fat or oil suspended in an agar base. To this oil emulsion agar various indicators may be added to aid in observing the changes that occur in the fat globules. The indicators fall into four groups: those indicating changes in pH; those measuring changes in Eh; those considered to be specific for fatty acids; and those that unite with the fatty acids, forming coloured substances such as the blue soaps formed when solutions of certain copper salts are added. Even without the addition of indicators, sufficient changes occur in the texture of some types of fat globules to indicate what is considered to be lipolytic action. The mechanism and significance of some of these reactions have been discussed by the authors in previous papers (1, 2, 3).

In the preceding paper of this series (4), the hydrolytic and oxidative action of 40 species of bacteria on triolein was recorded. In this paper, observations have been made on the action of these same cultures on globules of triolein in an agar base, using several different indicators. Besides the 40 cultures of the previous experiments, additional strains of many species have been used.

Materials and Methods

The triolein used in these experiments was manufactured in England and procured through the British Drug Houses, Limited. The agar and the

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nutrients for preparation of the media were all Difco products. Unless otherwise mentioned, the dyes and indicators were standard B.D.H. products.

The media used throughout the experiments were prepared in a manner similar to that ordinarily used in the fat emulsion agar technique. One per cent of oil was added to an agar base and sterilized in the autoclave for 15 min. at 15 lb. pressure. In some media the indicator was added before sterilization; with others it was flooded over the surface of the agar after the plates had been incubated. After the sterilized medium had cooled sufficiently, it was shaken vigorously to disperse the fat in the form of fine globules and then poured into sterile plates. Inoculation was made by streaking the surface of the solidified agar with organisms from actively growing cultures. All examinations were made through the low power lens of the microscope, although in some cases changes were evident to the naked eye.

In the first series, each of the 60 cultures were used to inoculate each of the following media:

- (1) Triolein + plain agar (no added nutrients) + methylene blue
- (2) Triolein + plain agar (no added nutrients) + Nile blue sulphate
- (3) Triolein + standard beef extract agar + methylene blue
- (4) Triolein + standard beef extract agar + Nile blue sulphate
- (5) Triolein + standard beef extract agar

The dyes were added as aqueous solutions in amounts to make their concentration in the medium 1 : 40,000. The plates to which dyes were not added were prepared in quadruplicate. These were later flooded with (a) a saturated solution of copper sulphate, (b) a 0.4% solution of *p*-aminodimethylaniline monohydrochloride, and (c) Schiff's reagent. The fourth set was left completely untreated.

All plates were incubated for 8 days at 25° C. (77° F.). Observations were made daily on all plates except those that were to be flooded on the eighth day.

This whole series of 480 separate tests was repeated three times.

General Observations on Changes Occurring in the Inoculated Globules of Triolein

Careful microscopical observations demonstrated a remarkable variation in the form and colour of the disintegrating fat globules. Continued observation showed that many of these variations were fundamentally the same, being different stages in a few general types of breakdown. Among the factors influencing the rate of change in the globules, are their size (the smaller they are, the faster they change), their position in relation to the bacterial colony, and their relation to the surface of the medium. Many organisms produce changes, in the globules surrounding the colony, that differ markedly from the changes in globules in actual contact with the bacteria; some cause changes only in the globules that are in actual contact with the colony.

Brief descriptions only of the main types of changes that occur in the globules will be given; the results of the action of individual cultures on the

globules will be tabulated in terms of these changes. They are subdivided into changes in colour and changes in form and texture of the globules. These relate specifically to triolein, but, in general, occur in a similar way in most other fats.

CHANGES IN COLOUR AND FORM

Normal Blue

This refers to globules in media containing either Nile blue sulphate or methylene blue. The first dye stains normal globules of triolein from pink to rose; the second does not stain them. As lipolysis proceeds, those stained with Nile blue sulphate gradually change through shades of mauve and purple to deep blue. With methylene blue they simply become uniformly blue. As can be seen in Table I, these colour reactions parallel the formation of

TABLE I

REACTIONS OF GLOBULES OF TRIOLEIN INOCULATED WITH PURE CULTURES OF BACTERIA, WITH SEVERAL DIFFERENT INDICATORS

Name of organism	Number of strains used	Colour changes							Changes in form		
		Blue, N.B.S.	Blue, M.B.	Blue droplets, M.B.	Red, di-methyl dye	Blue, di-methyl dye	Brown, di-methyl dye	Blue, C.S.	Projections	Crystals	Decolorization and granulation
<i>Pseudomonas aeruginosa</i>	5	+++	++	-	+	+	+	+	+	+	+++
<i>fluorescens</i>	2	+++	++	-	+	+	+	+	+	+	+++
<i>mucidolens</i>	1	+++	++	-	+	+	+	+	+	+	++
<i>fragi</i>	1	+++	++	-	+	+	+	+	+	+	++
<i>Alcaligenes lipolyticus</i>	1	+++	++	-	+	+	+	+	+	+	+
<i>viscosus</i>	2	+++	++	-	-	-	+	+	+	+	-
<i>faecalis</i>	2	-	-	-	+	-	-	-	-	-	-
<i>Achromobacter lipolyticum</i>	1	++	++	-	-	+	+	+	+	+	-
<i>Phylomonas campestris</i>	1	++	+	-	+	+	-	+	+	+	+
<i>Bacillus subtilis</i>	1	-	-	+	-	-	-	-	+	-	-
<i>mycoides</i>	1	+	+	+	-	+	-	+	+	-	-
<i>panis</i>	1	-	-	+	-	-	-	-	+	-	-
<i>cereus</i>	1	+	+	+	-	+	-	+	+	-	-
<i>graveolens</i>	1	-	-	+	+	-	-	-	+	-	-
<i>mesentericus</i>	1	-	-	+	+	-	-	-	+	-	+
<i>Staphylococcus citreus</i>	1	-	-	-	-	-	+	-	+	-	-
<i>aureus</i>	1	++	++	+	-	-	+	+	+	+	-

NOTE: (+++) = strong reaction; (++) = moderate reaction; (+) = weak reaction; (-) = no reaction.

N.B.S. = Nile blue sulphate; M.B. = methylene blue; C.S. = copper sulphate; di-methyl dye = *p*-aminodimethylaniline monohydrochloride.

blue soap in similar plates flooded with saturated copper sulphate solution. By comparing organisms bringing about this "normal blue" reaction with those organisms shown in the preceding paper of this series (4) to be capable of hydrolysing triolein, its significance is at once apparent. This normal blue colour in globules stained with methylene blue, Nile blue sulphate, or flooded with copper sulphate solution is indicative of hydrolysis of the fat. In previous papers (1, 3) the authors have shown that besides methylene blue, many other dyes can replace the Nile blue sulphate, the results being the same. Contrary to general belief, these colour reactions are not similar to those obtained with ordinary pH indicators, but are brought about by the extreme solubility of the oxidized form of many Eh indicators in fatty acids and the tendency of fatty acids to oxidize these dyes.

The enzymes bringing about hydrolysis, as indicated by these colour reactions, are able to diffuse through the agar to affect globules remote from the colonies. With strong lipase formers, such as some of the members of the genus *Pseudomonas*, or *Staphylococcus aureus*, this area of diffusion may extend between 1 and 2 cm. beyond the colony.

False Blue

This has been observed only in media containing Nile blue sulphate and then, only when the medium was lacking all nutrients other than fat. The globules surrounding the colony change from pink to delicate shades of blue or bluish-green. It usually occurs only after 5 or 6 days' incubation. Apart from the unusual conditions under which it occurs, it is not likely to be confused with the normal reaction of lipase formers with this dye. No matter how long the incubation continues the colour remains a delicate shade, in marked contrast with the intense dark purples and blues in globules undergoing hydrolysis.

This false blue was observed in cultures of *Escherichia coli* and *E. coli communior*, *Aerogenes cloacae*, and the following members of the *Salmonella* genus, namely, *S. schottmülleri*, *aertrycke*, *columbensis*, *hirschfeldii*, *morganii*, *suipestifer*, *paratyphi*, and *enteritidis*. It did not occur with three strains of *S. pullorum*.

This colour reaction appears to be owing to the reducing activity of the bacteria concerned. Castell and Bryant (2) have shown that a blue colour in globules stained by Nile blue sulphate may be derived from either of two sources; it may be caused by the absorption of the aqueous blue portion of the dye when fatty acids or some other similar substance are present in the fat or it may be produced by a *reduction* of the pink fat-soluble portion of the dye. The tests for lipolysis in the preceding paper indicated that these organisms did not hydrolyse triolein and therefore the blue colour could not be caused by the presence of fatty acid in the globules. On the other hand, tested with *p*-aminodimethylaniline monohydrochloride and similar indicators, not only are the colonies of these organisms oxidase-negative, but they have a reducing action on these dyes in areas near those containing the false blue

globules. These organisms are also known to produce a relatively low potential in media in which they are growing. It is most probable then, that the blue in these globules is the result of the strong reducing activity of these particular bacteria.

Blue Droplets (Fig. 1, Plate I.)

These appear to occur only in media containing methylene blue. Instead of becoming uniformly blue, the globules contain minute blue droplets. These frequently increase in size until they occupy a large portion of the globules. The blue liquid appears to be insoluble in the fat, but readily diffuses into the agar when it comes in contact with it. These occur only in globules that are in contact with the bacteria. They were present in plates containing the following aerobic spore formers: *B. subtilis*, *B. graveolens*, *B. cereus*, *B. panis*, *B. mycoides*, and *B. mesentericus* and they were present, though not very abundant, in plates of some of the cocci. In some instances, organisms that were lipolytic showed indications of the formation of these droplets but they always became obscured as hydrolysis progressed in the globule. Table I shows only those cultures that produced the droplets not obscured by subsequent lipolysis.

The presence of these "blue droplets" in the globules in media to which methylene blue was added, raises several questions apart from their identity. Methylene blue is not generally considered to be soluble in fat. How then, does the colour get into the isolated little drops in the interior of the globule? It was observed that if the droplets came in contact with the agar they readily diffused out of the fat so that this could not be the source of the stain. The possible explanation may be contained in an observation of Michaelis (6) "Even with methylene blue, it is somewhat disturbing that the free base is very difficultly soluble". It may be that small particles of this reduced dye are suspended in the fat before the agar has solidified. Castell and Bryant (2) have previously noted that certain fat-insoluble acids (such as lactic acid) when mixed with the fat and shaken with reduced methylene blue, were able to oxidize the dye. At that time it was predicted: "peroxides and some of the carboxy acids might be the cause of isolated blue droplets in globules. As they are not fat-soluble, the blue they abstract will not diffuse throughout the whole globule but remains in isolated areas". Incidentally, it has been observed by the authors that these blue droplets are much more abundant and occur in cultures of a great many more species of bacteria when the triolein has been replaced by butterfat or certain other natural fats and oils.

Pseudo-oxidation Reactions

These occur in plates flooded with Schiff's reagent or *p*-aminodimethylaniline monohydrochloride. These dyes in their reduced or colourless form are insoluble in fat but are readily soluble when they are oxidized to a red colour. If a solution of the reduced dye is poured over an inoculated plate, certain colonies readily oxidize the dye, rendering it fat-soluble, with the result that globules surrounding these colonies soon become coloured. This occurs

in cultures of all those organisms previously shown to be "oxidase-positive", and to some extent in cultures of some other organisms. Although it has been shown that there is a close correlation between those organisms that oxidize triolein and those that are oxidase-positive, it appears that in this colour reaction the bacteria act on the dye rather than on the fat. As the dye solution in other portions of the plate becomes slowly oxidized by the atmosphere, all the globules become coloured in a similar way. It would appear that the addition of fat globules to the media used for detecting "oxidase"-producing organisms would enhance the delicacy of the test.

Apart from their pseudo-oxidation reactions, both of these indicators have shown other colour changes in globules acted upon by lipolytic colonies. The Schiff reagent produces a darker purplish-red colour, the dimethyl indicator—yellow, brown, black, blue, or green. The significance of these colour variations has been treated in a separate paper (1). It is sufficient to observe here that they all appear to be associated with fatty acid production.

Decolorized Globules and Granulation

This refers mainly to globules in media containing either Nile blue sulphate or methylene blue and occurs only in globules that are in immediate contact with the bacteria. With some lipolytic organisms, after the deep blue stage has been reached, further activity completely removes the colour. This always appears to be accompanied by changes in the texture of the globules. From a liquid or semi-liquid, they change into an amorphous granular material. The size of the particles and their form varies considerably, but they are characterized by the absence of anything similar to the typical long thin fatty acid crystals or the feathery crystals of fats (Fig. 7).

The most pronounced reaction is produced by the strongly oxidase-positive organisms, especially strains of *Pseudomonas aeruginosa* and *P. fluorescens*. In contrast, globules acted on by less strongly oxidase-positive bacteria, such as *Alcaligenes lipolyticus* or *Achromobacter lipolyticum* retain the blue colour for a much longer period and those in cultures of oxidase-negative *Alcaligenes viscosus* and *S. aureus* retain the blue colour indefinitely.

When the non-lipolytic, oxidase-positive and oxidase-negative organisms were first examined they apparently showed little action on the globules as compared to those described above. However, many of the plates were examined more than two weeks after inoculation and in cultures of all oxidase-positive organisms and some that were oxidase-negative a somewhat similar granular material was present. The oxidase-negative group consisted mainly of the Gram-negative rods. The reaction did not occur in cultures of cocci nor bacilli except *B. mesentericus*. It is significant that the extent of these reactions corresponds closely to the oxidation reactions determined by chemical tests in the preceding paper. One noticeable difference, however, is that where lipolysis occurs, this apparent oxidation reaction occurs in the globules very much earlier and more vigorously.

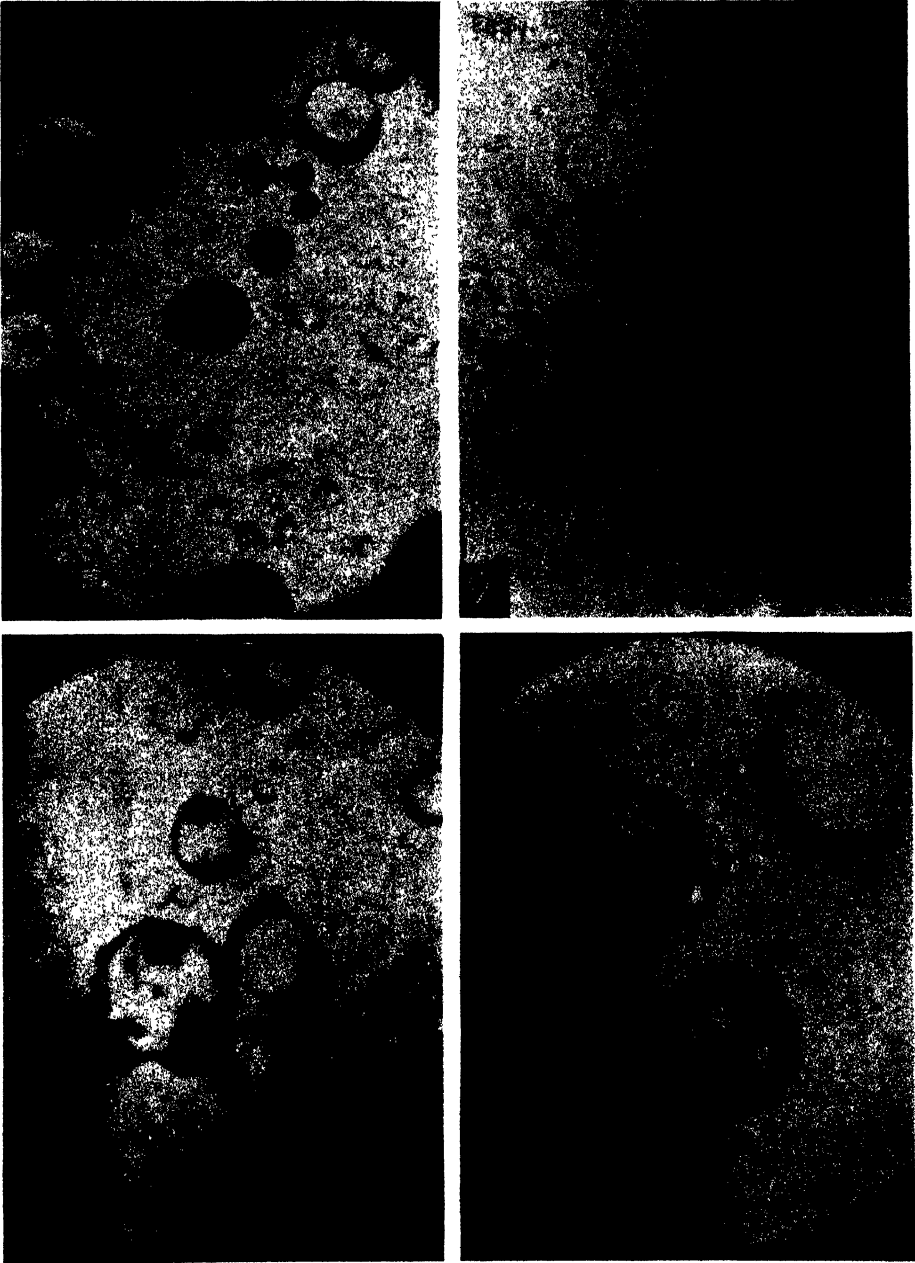


FIG. 1. "Blue droplets" in a large surface globule of triolein in a medium containing methylene blue and inoculated with *B. graveolens*. $\times 180$.

FIGS. 2-4. "Projections" on globules of triolein. Fig. 1 shows general appearance of globules close to, but not touching the colony. $\times 60$. Figs. 3 and 4 show the crystals in greater detail. The main portion of these globules did not give any of the ordinary reactions for fatty acids. $\times 180$.

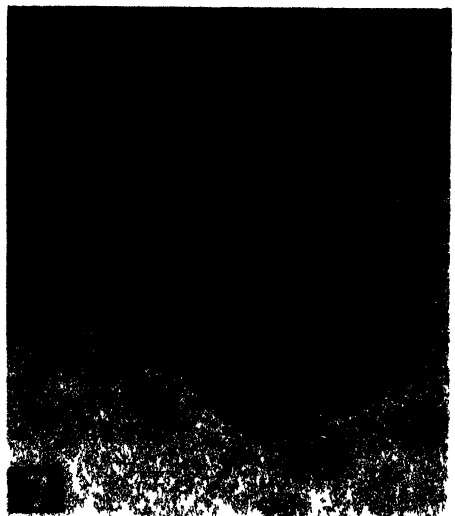
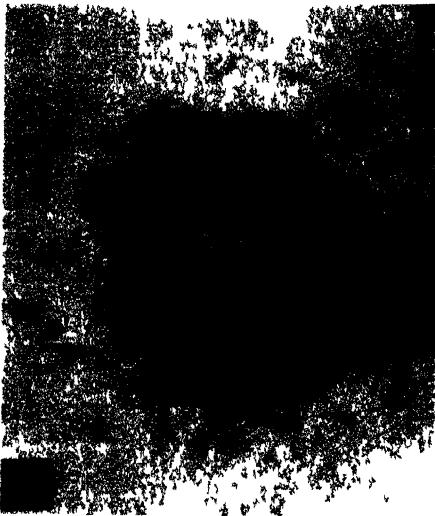


FIG 5 Edge of colony of *P. fluorescens* and adjacent, decomposing fat globules showing typical fatty-acid-like crystals. Note the marked tendency of the crystals to grow out into the medium. $\times 80$

FIG 6 Decomposing fat globule deep in the medium under a colony of *A. viscosus*. Many of the acid-like crystals have formed in the agar away from the globule. $\times 160$

FIG 7 Granulation of globules of triolein which have been covered with a colony of *P. fluorescens*. $\times 80$.

Several series of tests were made substituting oleic acid for the triolein. It was found that the oxidase-positive bacteria brought about rapid changes in the acid globules and that similar, though frequently much less change, was caused by some of the oxidase-negative, Gram-positive organisms. The least change was caused by members of the bacillus and the coccus groups.

Projections (Figs. 2-4, Plate I)

These changes are more easily observed than they are described. The chief characteristic is the formation of peculiar protrusions or swellings that form on the exterior of the globules. They are composed of radiating crystals, sometimes loosely, and sometimes tightly packed. They are coloured blue by Nile blue sulphate, methylene blue, or copper sulphate solutions. Occasionally they give a sharp colour reaction with these indicators while the globule to which they are attached remains unchanged. They are formed on globules both in contact with and away from the colonies.

As well as occurring in all cultures of lipolytic organisms, they appeared also on globules in the plates containing each of the bacilli tested, and occasionally in plates of some of the non-lipolytic cocci (*Sarcina aurantiaca* and *Staphylococcus citreus*). Incidentally, it was noted that some of the common varieties of actinomycetes that were contaminants on discarded plates, produced these formations in a very marked manner without apparently having any other effect on the fat.

No explanation can be offered concerning the nature of these crystals other than that they are acid in reaction and appear to be extremely similar to crystals of myristic acid prepared by mixing the acid in a matrix of triolein and dispersing it as globules in an agar medium. (See *photomicrographs in* (2).)

Acid Crystals (Fig. 5, Plate II)

Many globules undergoing hydrolysis, as indicated by the dye reactions, produced a wide variety of needle-like crystals. These usually protruded out into the agar from the disintegrating globule, but occasionally were observed scattered in the agar surrounding the globules as though they had formed from some substance that had diffused away from the fat (Fig. 6, Plate II). In some instances it was difficult to distinguish these from the preceding type of crystal. However, the so-called acid crystals invariably were accompanied and preceded by hydrolysis of the fat as indicated by the dye reactions. The best samples of these crystals were found in cultures of *S. aureus* and *A. viscosus*. They were present, however, in all cultures of lipolytic organisms and in none of the non-lipolytic bacteria. The significance of these crystals, like those of the preceding type, can only be inferred. Their general appearance, their tendency to protrude out into the agar from the globules, and their reaction to copper sulphate, Nile blue sulphate, and other dyes and indicators all strongly suggest that they are fatty acids. How solid fatty acids could be formed by hydrolysis of pure triolein or the products of this reaction is a matter of conjecture. It may be mentioned, however, that by flooding the

plates with suitable oxidation-reduction indicators, these crystals are formed only in a reducing environment.

EFFECT OF ADDED NUTRIENTS ON LIPOLYSIS

With one or two probable exceptions there was evidence of growth on all the plates to which nutrients had been added. On plain agar, even after 20 days' incubation, the growth was meagre, or in many cultures, absent. Most of the organisms having a marked action on the fat in plates containing nutrients, showed a similar but very limited action on the fat in the plates containing only plain agar. As was mentioned before, the "false blue" reaction occurred only in those plates containing plain agar.

REACTION OF OTHER Eh INDICATORS IN GLOBULES OF TRIOLEIN INOCULATED WITH BACTERIA

In previous papers, Castell and Bryant (2) and Castell and Garrard (3), have shown that many common Eh indicators react like methylene blue inasmuch as, although they are not fat soluble, they are readily soluble in fat containing small percentages of fatty acids. Triolein agar plates previously inoculated and incubated for 48 hr. were flooded with seven of these dyes. The dyes were used as 1% aqueous solutions. Shortly after flooding, the plates were washed off with water and the globules examined microscopically.

Of the four organisms used for inoculation, two were lipolytic and two were not and one out of each of these pairs had a strong oxidizing action on the fat. From the results of these observations (Table II) it is apparent that these particular oxidation-reduction indicators react like methylene blue. Coloration of globules surrounding a colony indicates hydrolytic action and has nothing to do with the oxidizing activities of the bacteria.

TABLE II

ABSORPTION OF Eh INDICATORS BY GLOBULES OF TRIOLEIN THAT WERE PREVIOUSLY ACTED UPON BY BACTERIAL CULTURES

Dye solution	Colour in the globules			
	<i>A. lipolyticum</i>	<i>P. fluorescens</i>	<i>B. mesentericus</i>	<i>Diplococcus capsulatus</i>
Malachite green	Blue	Blue	Colourless	Colourless
Methyl violet	Red	Red	Colourless	Colourless
Indigo carmine	Colourless	Colourless	Colourless	Colourless
Basic fuchsin	Red	Red	Colourless	Colourless
Neutral red	Red	Red	Colourless	Colourless
Rose bengal	Red	Red	Colourless	Colourless
Gentian violet	Purple	Purple	Colourless	Colourless
Methylene blue	Blue	Blue	Colourless	Colourless

Discussion and Summary

The observations described in this paper indicate that bacteria bring about several distinct changes in globules of triolein. By comparing these changes and the organisms that produce them with the results of chemical tests reported in the preceding paper it is apparent that one group of changes is associated with lipolytic organisms and the other with bacteria that oxidize either the fat itself or the product of its hydrolysis. Some organisms, especially members of the *Pseudomonas* genus are able to bring about both these reactions.

The action of lipolytic bacteria in globules of triolein is indicated, first by their intense colour reaction when stained by Nile blue sulphate, methylene blue, and other Eh indicators or saturated copper sulphate solution, and second, by the formation of fatty-acid-like crystals. The oxidizing bacteria decolorize stained fat that has been previously hydrolysed and at the same time change the globule from a liquid or semi-liquid to granular material. A somewhat similar, but retarded and much less extensive change occurs in globules not previously hydrolysed.

These observations appear to be in general agreement with those on the bacterial action on fat as described by Frazier (5): "The chief action of bacteria on fats is a hydrolytic cleavage by means of lipase, in which the fats are split into glycerine and fatty acids. Glycerine is readily acted on by a number of bacteria and is usually the first of the split products to be attacked. The course of the fermentation of glycerine will depend upon the organisms present and the general conditions but it is generally changed into lactic acid, volatile acids, and aldehydes. The fatty acids which result from the hydrolysis of the fats or the fermentation of the glycerine are usually not attacked until the more available glycerine is destroyed. Then, however, these acids may be further split by some types of bacteria into simpler acids or to CO_2 and water. The first part, then, of the action of bacteria on fat is usually hydrolysis and this is followed by oxidation processes".

Several false colour reactions, apparently indicating lipase action, have been observed and the conditions described under which they occur.

Observations have also been made on other types of change occurring in globules caused by bacterial activity; their significance is still unexplained.

It is interesting to note that there are two general "spheres of influence" in which the same bacterial colony may affect globules in an entirely different manner. These are the areas out around or deep under the colony, and the section immediately in contact with the bacteria. Many changes, including those considered to be oxidation reactions, occur only when the bacteria are in contact with the globules, whereas all those considered to be associated with lipolysis can occur in globules a considerable distance away from the nearest organism. This would suggest that one type is caused by a diffusible enzyme (lipase) and that the other is a reaction occurring at the cell surface. It is, further, interesting to note that when certain oxidation-reduction indicators are flooded over these colonies, the area in the medium where lipolysis

occurs reduces the dye but it is oxidized by contact with the colony itself. This is shown by a coloured colony surrounded by a clear zone in the medium. Similar dyes poured on colonies (*S. aureus*, *A. viscosus*, etc.) result in a colourless colony surrounded by an area of reduction.

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ENVIRONMENTAL REACTION OF PHYSIOLOGIC RACES OF *PUCCINIA TRITICINA* AND THEIR DISTRIBUTION IN CANADA¹

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Abstract

Studies were made of the effect of temperature and light on the reactions to leaf rust (*Puccinia triticina* Erikss.) of the differential varieties of wheat used for the identification of physiologic races of this rust. With a large number of races both temperature and light were found to exercise a marked influence. The reactions of all varieties were not, however, influenced in the same direction. With lower temperature, Malakof and Democrat became increasingly susceptible, while Carina, Brevit, and Hussar became increasingly resistant. Webster and Mediterranean did not react consistently in either direction, and Loros was but little influenced by temperature. All of the differential varieties showed a more or less marked tendency to become increasingly resistant under conditions of short day length and weak light. In general, more pronounced changes in reaction were produced by variation of temperature than of light.

Surveys for the distribution of physiologic races of leaf rust in Canada were conducted annually since 1931 with the exception of the years 1932 and 1935. Forty-nine races were identified. Most of the prevailing physiologic races were found to be widely distributed throughout the country. Evidence was, however, secured that certain races were largely confined to certain areas. Races 1, 58, 76, and 81 were common for several years in Eastern Canada but were not encountered in the Prairie Provinces until 1940 when one collection of each of the three first-mentioned was made in that area. Races 11 and 53 were largely limited to British Columbia and the adjacent province of Alberta. Races of wheat leaf rust have undergone no marked change in respect to identity or relative prevalence in the last few years in Canada.

Introduction

Although studies on the specialization of wheat stem rust (*Puccinia graminis Tritici* Erikss. & Henn.) have been conducted in Canada since 1919, no attention was given to leaf rust (*Puccinia triticina* Erikss.) until 1931. There were two main reasons for this: (1) the much greater destructiveness of stem rust demanded that attention be first given to that rust, and (2) the fact that the varieties of common wheat grown in Western Canada until about 1930 were not highly susceptible to leaf rust. The introduction of highly susceptible varieties such as Kota and Ceres, resulted in a decided increase in the economic importance of this rust. The realization by plant breeders that the new wheat varieties that they were engaged in producing must possess resistance to leaf rust as well as to stem rust before they could be accepted as permanent substitutes for the older varieties, made it imperative that a study be made of the pathogenic characteristics of the leaf rust organism.

Mains and Jackson (7), in 1926, laid a foundation for studies on the specialization of the leaf rust organism by selecting a group of 11 wheat varieties

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by means of which they were able to demonstrate that the rust was specialized into many physiologic races. These eleven varieties were later reduced by Johnston and Mains (6) to eight, namely, Malakof (C.I. 4898), Carina (C.I. 3756), Brevit (C.I. 3778), Webster (C.I. 3780), Loros (C.I. 3779), Mediterranean (C.I. 3332), Hussar (C.I. 4843), and Democrat (C.I. 3384). These varieties, which have since gained general acceptance as differential hosts for leaf rust, were used in the identification of physiologic races of leaf rust by the writers in 1931. The variety Thew, used as a differential host by Waterhouse in Australia, was included with these varieties in the 1940 rust survey. As it proved susceptible to all collections of leaf rust made in that year, as well as to races 27, 28, 41, 65, 71, and 103, it is probably not a suitable variety to differentiate North American leaf rust races.

It soon became evident that the identification of these races was by no means a simple and easy task. The frequent presence of several physiologic races in a single collection of rust made it necessary to separate the races by propagating each culture of the rust from single uredial pustules or even single urediospores. A more serious difficulty, however, was the apparent instability of the host reaction of certain of the differential varieties. Frequently it was found that races identified without difficulty and put aside in storage would, when again tested after an interval of a few months, produce infection types considerably at variance with those produced when the race was originally identified. For example, races originally identified as race 13 or 20 were occasionally reidentified, under different environmental conditions, as race 31. In these instances the difficulty was obviously caused by instability of the reaction of the varieties Carina and Brevit. Similar difficulties were frequently experienced also with the reaction of the variety Hussar, the instability of which made it sometimes difficult to distinguish such races as 9 and 31 or 2, 15, and 34.

The apparent instability of the host reaction of these and certain other varieties led to a decision to investigate the effect of certain environmental conditions, particularly temperature and light, on the host reactions of the differential varieties used for the identification of physiologic races of leaf rust. The present paper attempts to give an account of this work as well as a summary of the physiologic race surveys thus far conducted in Canada. A summary of the physiologic races identified by the writers up to 1936 has already appeared in an earlier publication (8).

The Effect of Temperature and Light on the Reaction of Differential Varieties

Before the work here described was undertaken, it had been shown that environment was capable of influencing the host reaction of certain of the differential varieties. Mains and Jackson (7) stated that Hussar showed considerable variation in its reaction to some physiologic races, often being resistant in fall or winter but only moderately or slightly resistant in late spring. Waterhouse (12) noted that Carina and Hussar showed flecks in

the winter months and a "4" infection type in summer when infected with Australian race 1. Dodoff (1) stated that the reactions of Brevit were apparently influenced by temperature, and Gassner and Straib (2) showed that the varieties Malakoff, Norka, Democrat, and Mediterranean became increasingly susceptible with lower temperatures.

The Effect of Temperature

It is clear from the above statements that the environment is capable of affecting host reactions to a considerable degree. Only in the work of Gassner and Straib, however, had it been shown conclusively that a particular environmental factor (temperature) was responsible for a pronounced effect on host reaction. Gassner and Straib (2) showed that the reactions of Malakoff and Norka to race 14 varied from infection type "0" at 18.7° C. to types "3" and "4" at 6° C., and of Democrat from types "0" or "1" at 18.7° C. to types "3" and "4" at 16.6° C. and lower temperatures. As the varieties Webster and Similis produced the same reaction at 18.7° C., 16.6° C., 10.9° C., and 6.0° C., the response to low temperature was clearly not characteristic of all varieties resistant at ordinary temperatures.

That high temperatures can produce a comparable modification of host reaction was shown by Johnson and Newton (5) who found that at temperatures above 85° F. (29° C.) Little Club wheat became resistant to physiologic races to which it is susceptible at ordinary temperatures.

While the present work was in progress, further light was thrown by other investigators on the influence of temperature on the reactions of differential varieties. Roberts (9) stated that the effects of low temperature and low light intensity were similar—both having a tendency to increase resistance of varieties normally susceptible. Varieties normally resistant showed increased susceptibility with increased temperature and light intensity. Lacking facilities for control of temperature, she experienced difficulties in determining the relative importance of temperature and light in changing the varietal reactions.

Hassebrauk (3) showed that low temperatures (about 6° C.) generally increased susceptibility, though the reverse was true for some varieties. The varieties Carina and Brevit formed an exception in that they were more resistant at low temperatures.

Experiments conducted by the present writers on the influence of temperature on the reaction of the differential varieties to eight physiologic races of leaf rust are summarized in Table I. The tests were carried out in two different series of experiments, one conducted in April, 1936, the other in February, 1939. In both instances, the plants tested at the highest and lowest temperatures were kept in sections of the greenhouse with thermostatic temperature control. The plants tested at the medium temperature were kept in a section of the greenhouse in which temperature fluctuation was kept within narrow limits by manipulation of the steam pressure and the ventilators.

Thermographic records showed that fluctuations of temperature in this section were only slightly greater than in the sections with thermostatic temperature control.

TABLE I

INFECTION TYPES ON DIFFERENTIAL VARIETIES OF *Triticum vulgare* PRODUCED BY EIGHT PHYSIOLOGIC RACES OF *Puccinia tritici* AT THREE CONSTANT TEMPERATURES

Physio- logic race	Temp., °F.	Date of expt.	Wheat variety								Race identified
			Mala- kof	Carina	Brevit	Web- ster	Loros	Mediterranean	Hussar	Democrat	
9	57	Feb. 1939	4=	2-	1-	4-	4=	x-	x+	3+	No known race Race 9 Race 20
	64		3+	2+	2	3+	3+	1±	2±	1-	
	69		3+	3+	3+	4-	3+	1±	3	1-	
			4	1-2	1-2	4	4	0-1	1-2+	0-1	
11	57	Feb. 1939	x+	x+	x+	x	3+	3+	x	3±	No known race No known race Race 66
	64		0	x+	3	3=	3+	2	2-	1	
	69		0	3	3+	x	3+	1	3	1-	
			0	2+	3-4	1-2+	3-4	1-2	0-2	0-2	
15	60	Apr. 1936	1-	1-	1	0	1-	3	2-	3	Race 15 Race 15 Race 62
	65		0	1-	1-	0	1-	3	2-	3	
	75		0	1-	3	1-	1-	3+	3	3+	
			0	0	0-1	0	0-1	4	0-1	4	
31	60	Apr. 1936	3	1+	1-	3+	3+	1-	3	x	Race 27 Race 31 Race 20
	65		3+	2+	2-	3	3+	1-	3	2-	
	75		3+	3+	3+	3+	3+	1-	3+	1-	
			4	2	1-2	4	4	1-2	3-4	1-2	
41	57	Feb. 1939	4	2±	1+	4-	4-	x	3	3+	No known race Race 41 Race 77
	64		4-	3+	2++	4-	4-	1+	2++	3±	
	69		4-	3+	3+	4-	3+	3±	3+	3+	
			4	2-3	2	4	4	2	2	3-4	
44	57	Feb. 1939	3+	1	1±	1-	3	x-	x-	3+	No known race Race 44 Race 58
	64		0	1±	2+	1-	3	x-	1-	3	
	69		0	1+	3	0	3	3	2+	3	
			0	2	2	0-2	4	x	0-1	4	
87	60	Apr. 1936	1-	3	3+	3	3+	x	2-	x	No known race Race 87 No known race
	65		1	3+	3+	3	3+	x	2	x	
	75		0	3+	3+	2-	3+	2-	3+	1+	
			1	4	4	3	4	x	2	x	
88	60	Apr. 1936	1-	x-	2	3+	x	x	1	3+	No known race Race 88 Race 12
	65		1	x	x	3	3+	x	1	3+	
	75		0	3+	3	1	3+	3+	3	3+	
			1	2-3	2-3	3	4	x	1	4	

Figures in bold-face represent the infection types recorded for type race in key prepared by H. B. Humphrey, C. O. Johnston, R. M. Caldwell, and L. E. Compton.

The plants in each series of experiments were exposed, as nearly as possible, to the same conditions of light. Inoculations of plants kept at all three temperatures were done on the same day. Light conditions at the different temperatures were not, however, absolutely identical because rust developed most rapidly at the highest and most slowly at the lowest temperature; the result was that the uredia, at the highest temperature developed about two days earlier than at the medium temperature and about seven days earlier than at the lowest temperature. Corresponding phases of rust development therefore took place under slightly different light conditions at the three temperatures.

The results of the experiments show clearly that there is a pronounced varietal response to temperature. The varieties Malakof and Democrat showed increasing susceptibility with lower temperature. The reaction of Webster to most of the races was not greatly influenced by temperature. To some races, however, this variety proved susceptible at 65° F. and resistant or moderately resistant at 75° F. The varieties Carina, Brevit, and Hussar showed increasing susceptibility with higher temperature. The behaviour of the variety Mediterranean was rather inconsistent, its susceptibility to certain races tending to increase with lower temperature, whereas to other races its susceptibility was greatest at the highest temperature. The reaction of the variety Loros did not seem to be affected by temperature.

The results of tests with Malakof and Democrat confirm the findings of Gassner and Straib (2). They obtained, however, no indication of any pronounced response in the reaction of Webster to temperature. The response to temperature of Carina and Brevit agrees with that reported by Hassebrauk (3) who stated that these two varieties were more resistant at low than at high temperatures. The temperature response of Hussar is in agreement with results reported by Mains and Jackson (7), Waterhouse (12), and Roberts (9), who agree that this variety tends to be more resistant in the winter months than in the summer.

An examination of Table I may give the impression that the increased susceptibility of Malakof at low temperatures applies only when this variety is infected with certain races. It should be pointed out, however, that the mean temperatures for the low temperature tests were lower in the experiments of February, 1939, (57° F.) than in those conducted in April, 1936 (60° F.). This difference in temperature, though slight, may possibly account for the difference in reaction of Malakof, which retained its resistance at all temperatures in the earlier series of experiments but became susceptible at the lowest temperature in the later experimental series. Controlled experiments and general experience alike indicate that greater fluctuations of temperature are required to influence the reaction of Malakof than the reactions of Carina, Brevit, and Hussar.

It is clear from the evidence presented in Table I that temperature must be taken into consideration in the identification of physiologic races. In several instances the same culture of rust appeared to be a different physiologic

race at each of the three temperatures employed. A culture originally identified as race 31 appeared to be race 27 at 60° F., race 31 at 65° F., and race 20 at 75° F.* In view of such results it seems probable that pathogenically similar strains of rust have not infrequently been described as different physiologic races merely because the environmental conditions differed in the places at which identifications were made or in the seasons in which the work was performed.

The Effect of Light

Most of the evidence relative to the effect of light on host reaction to leaf rust is somewhat indirect in so far as only rarely has the effect of light been clearly separated from that of temperature. It is highly probable that variation in light (intensity of light and duration of day length) played a part in the production of the pronounced differences in varietal reaction in winter and summer noted by several investigators. Mains and Jackson (7) observed that Hussar was more resistant to certain physiologic races in winter than in summer. Waterhouse (12) reported that the varieties Hope, Iumillo, Hussar, and Carina, which were susceptible to Australian race 1 in summer, showed an indeterminate "x" reaction in winter and that the variety Thew, also susceptible in the summer months, proved highly resistant in winter. Roberts (9) found in the reactions of the variety Webster to race 73 a seasonal variation that appeared to be related to intensity of light. Tests conducted by her at the beginning, at the middle, and at the end of October, produced reactions of "4", "3+", and "2+" respectively. Her next test, which was made in February, produced the "x" reaction whereas the type "4" reaction was again produced in March and in subsequent tests in June, July, and August.

Recently, a study was made by Hassebrauk (3) of the effect of light on the reaction of the differential varieties to several races of leaf rust. In his study, the plants, following infection, were subjected to two different daily periods of light—one set of plants being exposed to normal daylight, another to only three or four hours of light per day. In general, reduction of light had either no effect on host reaction or tended to increase resistance slightly. The varieties Carina and Brevit proved exceptional in that their responses to light varied according to the races to which they were tested. To certain races they proved slightly more resistant under conditions of reduced light whereas to other races they were decidedly more susceptible in reduced light.

Still more recently Hassebrauk (4) has studied the effect of darkness during various portions of the incubation period on varietal reaction to leaf rust. Varieties moderately resistant under normal light conditions showed, when kept in darkness from the second to the fourth day of the incubation period, a definite increase of susceptibility. Darkness from the fourth to the sixth day tended to retard pustule development without greatly influencing reaction.

* Chester and Jamison (*Phytopath.* 29:962-967, 1939) have recorded somewhat similar difficulties in distinguishing between certain physiologic races. They regard races 9, 13, and 19 as environmental variants of the same race.

Darkness during the later portions of the incubation period generally increased resistance beyond that of control plants kept in normal light.

The possibility that seasonal variation in light intensity and daily duration of light might produce an important effect on the reaction to leaf rust of the differential wheat varieties led the writers, in the late winter and spring of 1936, to conduct experiments on the effect of light on host reaction. These experiments were carried out in two greenhouse sections with thermostatic temperature control; one section was maintained at a temperature of 60° F., the other at a temperature of 75° F. Three series of experiments were conducted: one in February (February 1 to 19), one in March (February 27 to March 12), and one in April (April 4 to April 17). These experiments are summarized in Table II, which shows the average reaction of six wheat varieties under conditions of approximately constant temperature but progressively increasing day length and light intensity. The increase in intensity of light in the greenhouse was considerably greater than out-of-doors owing to the presence on the glass, in February, of a coating of frost which had a noticeable shading effect.

In general, there was a tendency for susceptibility to increase somewhat as the day grew longer and light became more intense. This tendency was shown to a greater or less degree by all the varieties tested. Of the seven physiologic races used, all reflected the influence of increased light by their infection types on one variety or another. Several instances are recorded in Table II of a reaction, normally susceptible, being shifted towards resistance by low light intensity. Carina and Webster which are normally susceptible to races 10 and 87 tended, in February, to produce an indeterminate "x" reaction at 60° F., and Hussar, which normally is susceptible to race 52, showed an indeterminate reaction in March and a resistant reaction in February. Increased susceptibility with decreased light, such as reported by Hassebrauk (3), was not noted except perhaps in the reaction of Brevit to race 89 at 60° F.

Although light apparently induced some variation in normally susceptible reactions, the ones that responded most frequently were the reactions representing moderate or slight resistance, such as those described in the analytical key as "1" to "2", "2" to "3", or "x". Reactions ordinarily classified as "1" to "2" varied in some instances from "1" to "x", and reactions ordinarily indeterminate in some cases varied from 1 to 3. Evidently the reactions representing moderate or slight resistance are less stable than those representing high susceptibility or high resistance.

The fact that all tests were conducted at two temperatures makes possible a comparison of the relative influence on host reaction of light and temperature. In most instances, the maximum variation attributable to temperature was greater than that attributable to light. In some cases in which light showed no influence on reaction temperature exercised a profound effect. An example of such a temperature effect is shown in the reactions of Carina and Brevit to race 31, which were recorded as "1—" to "1+" at 60° F. and "3" to "3+" at 75° F.

TABLE II

INFECTION TYPES ON DIFFERENTIAL VARIETIES OF *Triticum vulgare* PRODUCED BY SEVEN PHYSIOLOGIC RACES OF *Puccinia triticea* AT A LOW, MEDIUM, AND HIGH LIGHT INTENSITY AND CONSTANT TEMPERATURES OF 60° F. AND 75° F.

Physio-logic race	Temp., °F.	Wheat variety															
		Carina				Brevit				Webster				Similis			
		Feb.	Mar.	April	Feb.	Mar.	April	Feb.	Mar.	April	Feb.	Mar.	April	Feb.	Mar.	April	Feb.
10	60° 75°	3 3	3- 3	3 3+	3- 3	3 3+	3 3+	3- 1+	3- 3	3 3+	3- 1+	3- 3	3 3+	3 3+	3 3+	3 3+	2- 3
31	60° 75°	1 3+	1+ 3	1+ 3+	1- 3	1- 3+	1- 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	1- 3+	1- 3+	3 3+	1+ 3
52	60° 75°	1 3	1- 3	1- 3	1 3	1- 3	1- 3	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+
85	60° 75°	1 3	1- 3	1- 3	1 3	1- 3	1- 3	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+
87	60° 75°	2+ 3	3+ 3	3+ 3	3 3	3+ 3	3+ 3	3- 3	3- 3	3- 3	3- 3	3- 3	3- 3	3- 3	3- 3	3- 3	3- 3
88	60° 75°	1 3	1- 3	1- 3	1 3	1- 3	1- 3	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+	3+ 3+
89	60° 75°	3 3-	3 3	3 3	3+ 3	3+ 3	3+ 3	3+ 3	3+ 3	3+ 3	3+ 3	3+ 3	3+ 3	3+ 3	3+ 3	3+ 3	3+ 3

Figures in bold-face represent the infection types recorded for type race in key prepared by H. B. Humphrey, C. O. Johnston, R. M. Caldwell, and L. E.

Distribution of Physiologic Races in Canada

The annual surveys of the distribution of physiologic races of leaf rust in Canada are summarized in Table III. These surveys have been carried out since 1931 with the exception of two years—1932 and 1935. Owing to the fact that all but one of the surveys (1934) covered practically the whole of Canada, it is possible to arrive at some conclusion as to the distribution of physiologic races in different parts of the country, and their persistence in those parts from year to year. In tabulating the results, the country was divided into four regions of which the agricultural areas are to a certain extent separated by natural barriers: first, the Maritime Provinces which are partially isolated from the remainder of Eastern Canada; second, the provinces of Ontario and Quebec; third, the Prairie Provinces of Manitoba, Saskatchewan, and Alberta, which are isolated from the farming areas of Ontario by great spaces of lake and forest; and fourth, British Columbia which is separated from the prairie region by the Rocky Mountains.

The surveys have shown that certain races are largely confined to certain regions and may be found to occur there annually whereas other races are widely distributed throughout the whole country. Races largely confined to Eastern Canada (Ontario, Quebec, and the Maritime Provinces) include races 158, 76, and 81. These have been collected year after year in the eastern provinces but have not been found in the prairie region of Western Canada except in 1940 when one collection of each of the three first-mentioned races was made in that area. The last three (races 58, 76, and 81) bear a rather close resemblance to each other pathogenically, and had perhaps better be regarded as biotypes of the same race rather than as three separate races.

There appear to be no well authenticated examples of races localized in the Prairie Provinces, but there is evidence of the localization of certain races in British Columbia. Race 11 had been collected 20 times since the surveys were begun, 17 times from British Columbia, twice from the adjacent province of Alberta, and once from the Maritime Provinces. Race 53 has been collected 11 times, 7 times in British Columbia, twice in Alberta, and twice in Manitoba. The occasional occurrence of the same races in British Columbia and in the adjoining province of Alberta suggests a limited amount of rust spread eastward either across the western mountain ranges from the coastal region, or northward from the Northwestern States. As the mountains seem to act as a barrier to the westward spread of rust, the latter assumption appears to be the more probable. At least, several races (races 5, 31, 44, 52) found commonly in the prairie region have never been collected in British Columbia.

Most of the predominant races, however, are not limited to any one region of the country. Races 2, 5, 9, 15, 31, 32, 34, 52, and 89 have been collected year after year both in Eastern Canada and the Prairie Provinces. The fact that these races were collected year after year would seem to indicate that,

TABLE III

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *P. triticea* FROM 1931 TO 1940.
(FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA.)

Physio- logic race	Maritime Provinces						Ontario and Quebec						Prairie Provinces						British Columbia						Total
	1931	1933	1936	1937	1938	1939	1940	1931	1933	1936	1937	1938	1939	1940	1931	1933	1936	1937	1938	1939	1940				
1	1			1	1	5	6			2	2			2		1									
2					4	4								3						1					
3					1	1								2											
5										2	2			5		1									
6														1											
9					2	1	1			1	1	4	1	1					1						
10																									
11															3										
13															1 ^a				3	6	5				
14															4										
15										4	3	3	3	3				2	4	6	2				
19								1							1	3		3		1	2	5			
20	2							1																	
27																		1	1						
28				1													1	1		1					
29					1	3								5				4		3	2				
30												2													
31		1									1	1	3			6		1	7	9	2	1			
32				1	4		3					1		1		3		2	1	1	3				
34		1				2	2			2	1	1	1	1		2		1	1			3			
35																									
37																									
39																									
41												1													
42																									
44		1		1				1		1	1					1									
50																		2							

NOTE: (a) = Collected in the province of Alberta; (b) = Collected in the province of Manitoba; (c) = Collected just north of Alberta boundary.

TABLE III—*Concluded*
DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *P. tritici* FROM 1931 TO 1940.
(FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA.)
—*Concluded*

Physio- logic race	Maritime Provinces						Ontario and Quebec						Prairie Provinces						British Columbia						Total
	1931	1933	1936	1937	1938	1939	1940	1931	1933	1934	1936	1937	1938	1939	1940	1931	1933	1936	1937	1938	1939	1940			
52	1							2	1			4		9	1									22	
53								1 ^a	1 ^a					1	1									11	
56	3	2						2	2												2			10	
58			1												1 ^c									16	
62																		1			1			1	
64																								1	
65																								1	
66																								5	
72																								2	
76																								1	
77																								50	
80			6	3			9								1 ^b				2					1	
81																								1	
83				4																				1	
85																								1	
87																								1	
88									1															3	
89			1																					2	
90									1	2					2			1			1	1		33	
101																								1	
103																		1						4	
104																								1	
	7	6	9	15	32	38	28	5	3	20	17	25	36	28	35	14	2	3	11	5	12	15		578	

NOTE: (a) = Collected in the province of Alberta; (b) = Collected in the province of Manitoba; (c) = Collected just north of Alberta boundary.

in respect to identity and relative prevalence, no radical change has taken place in this rust during the past few years.

Discussion

The difficulties involved in the identification of physiologic races of leaf rust appear to result chiefly from two causes: first, the high degree of specialization of the leaf rust organism and, second, the sensitivity of the reactions of certain of the differential wheat varieties to environmental influences. Because of the high degree of specialization, numerous pathogenically different strains of the rust are encountered each year, and, as the variations in pathogenicity are often slight, the investigator is frequently faced with the problem of deciding whether or not two or more pathogenically similar but not quite identical cultures should be regarded as one and the same race. As pointed out by Johnston and Mains (6) and Scheibe (10, 11), such pathogenic variants could almost certainly be distinguished clearly from each other by the addition of new varieties to the differential hosts now in use. Such a course would, however, in all probability have little practical value and would increase considerably the work involved.

Recognition of the second difficulty, namely, the responsiveness of the reactions of certain differential varieties to variations in temperature and light, has led investigators (2, 3, 9) to emphasize the importance of conducting such studies, as far as possible, under conditions of constant temperature and light. Most of the identification work, however, must perforce be carried out in greenhouses in which these factors can be controlled only to a limited extent. It is obvious, therefore, that a knowledge of the ways in which light and temperature influence rust reactions is of great importance. With such knowledge, allowance could be made for such influences at different seasons of the year. The experiments with temperature and light reported in this paper were designed to furnish such information. The results obtained, together with those previously presented by other investigators, make it possible to gauge to some extent the modification of host reaction induced by variation in these two important factors of the environment.

It seems clear from the experiments here reported and those of other investigators that the reactions of the various differential varieties respond differently to temperature. Malakof, Norka, and Demočrat tend to become increasingly susceptible with lower temperature. Carina, Brevit, and Hussar, on the other hand, become increasingly resistant with lower temperature. All of the differential varieties show a more or less marked tendency to become increasingly resistant under conditions of short day length and low light intensity. These facts, judiciously applied, should be of considerable assistance in the work of determining physiologic races by means of the differential varieties now in use. It would, of course, be more satisfactory to use as hosts varieties whose rust reactions showed little or no response to environmental conditions. The elimination, however, of the more reactive varieties, such as Carina, Brevit, and Hussar, and the substitution, as suggested by Hassebrauk (3) of other

more reliable varieties for them, if such could be found, might have the advantage of simplifying the work of identification, but, on the other hand, might have the disadvantage of making it difficult to relate the physiologic races identified by means of the new hosts with those identified in previous years by means of the discarded hosts. This difficulty would particularly apply if races identified in earlier years but now no longer available for study should reappear. With new differential hosts such races might be reidentified as wholly new races. In other words, a change of differential varieties might interfere with one of the important functions of physiologic-race surveys, namely, that of detecting the presence of new physiologic races.

The surveys for physiologic races carried out since 1931 indicate that races of wheat leaf rust have undergone no marked change in respect to identity or relative prevalence during this period in Canada, unless the appearance of race 76, in 1936, in the eastern provinces be regarded as such. As the surveys, prior to 1936, for leaf rust in Eastern Canada were rather inadequate, it is quite possible that this race was also present there in earlier years.

The reason for the localization of one or more races in certain areas is not altogether clear. Presumably a race, such as race 76, may overwinter in Eastern Canada or in some region to the south from which it may spread northward annually. The overwintering of certain races somewhere along the Pacific Coast and the obstruction to their eastward spread offered by the Rocky Mountains may perhaps account for the localization of those races in that region.

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THE DEVELOPMENT OF TWIN EMBRYO SACS, EMBRYOS, AND ENDOSPERM IN *POA ARCTICA* R. BR.¹

By V. ENGELBERT²

Abstract

The origin and development of twin embryo sacs especially in *P. arctica* R. Br. were studied microscopically in sections of whole spikelets representing consecutive stages in development from the time of emergence of the panicle until after pollination. Twin embryo sacs usually develop, one originating from the innermost of a row of four macrospores with the reduced chromosome number, the other from an aposporous cell that originates in the nucellus, near the chalaza and behind the normal archesporium. The individual development of the normal and aposporous embryo sacs from their respective mother cells is traced and the competition between them discussed. Both aposporous and normal egg cells develop parthenogenetically. Pollen germination appears to activate the aposporous polar cell to form endosperm which in turn nourishes the aposporous ($2n$) and (or) the "sexual" (n) embryo. The greatest number of plants originate from the aposporous embryo. A type of apospory was found in *P. alpina* L. from Greenland. The work of other investigators, especially on *P. pratensis* L. is reinterpreted in the light of these findings.

Introduction

In a recent publication, the author has made a report of breeding experiments and pollen tube growth studies, the results of which demonstrated apomictic reproduction and pseudogamy in the following species:

P. arctica R. Br. from West Greenland.

P. alpigena Fr. Lindm. from West Greenland.

P. alpina L. from West Greenland.

P. alpina L. from Georgian Bay, Ontario.

P. pratensis L. from Gaspé Peninsula, Quebec.

The present paper deals with those parts of the accompanying embryological studies that seem to the author to provide the information most essential for an interpretation of the *Poa* problem as a whole. This is believed to be the first study of flower biology and embryology of the Greenlandic species or biotypes of *Poa* and the first to trace to their proper origins the two embryo sacs found by many workers in *P. pratensis*, but not as yet explained.

The results in a way fulfil a prediction made by Nannfeldt in 1935³ (9) that the *Poa* case parallels Rosenberg's (10) *Hieracium* subgenus *Pilosella*, and suggest a reinterpretation of data supplied in some recent papers dealing with *P. pratensis*.

¹ Manuscript received December 30, 1940.

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Material and Methods

Culture

Seeds of *P. arctica* harvested in Greenland were used to raise the original plants. These seeds, which were chilled before seeding, had a lower percentage germination than those of the other species. To grow this high arctic species here at approximately $43\frac{1}{2}^{\circ}$ N. latitude and 300 to 400 ft. above sea level was found very difficult. Certain conditions had to be provided to keep the plants alive and sufficient material at hand for experiments. The species is narrowly restricted ecologically and does not withstand either desiccation or water surplus as does *P. alpigena*. It takes two years to raise *P. arctica* to flowering and to get suitable experimental material of the other species. Flowering occurred at the ordinary length of day found here in April and May, but it was found that *P. arctica* cannot be kept alive after flowering without being given a longer day. Continuous daylight was provided by a 60-Watt electric bulb suspended 28 in. above the plants. This produced good vegetative growth when other conditions were adjusted to keep a proper water balance and to eliminate the effect of high temperature.

Technique

Whole spikelets or whole panicles were fixed from two to several times a day from the time the top of the panicle began to emerge to several days after anthesis. Fixations were made by immersion for 1 min. in Carnoy's fluid then in La Cour's* 2BE for 24 hr.; later La Cour's 2BD was used as it seemed to give somewhat better results. Sections were cut $8\ \mu$ thick.

Staining was at first done with crystal violet, later with the Feulgen stain as formulated by Dr. L. C. Coleman**; the time of hydrolysis used was the one determined by Dr. B. B. Hillary***. Photomicrographs were made with a Leica camera attached to a Spencer microscope. An apochromatic 2 mm. (N.A. 1.30) oil immersion objective (90 \times) and a Leitz ocular (10 \times) were used for Plate I (A, B, C, D, and F). For Plate I-E, a dry 60 \times objective was used. Illumination was provided by a lamp designed, by Dr. D. H. Hamly†, after the Koehler arrangement. A combination of orange and green filters was used.

Embryology

DEVELOPMENT OF TWIN EMBRYO SACS

Origin of the two embryo sac mother cells

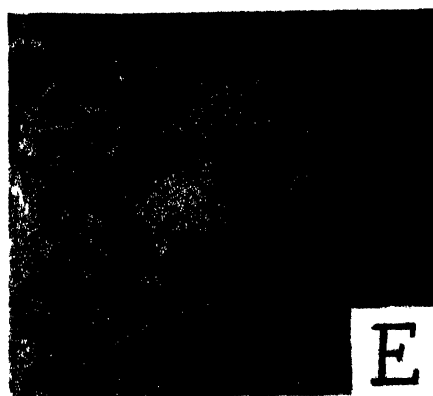
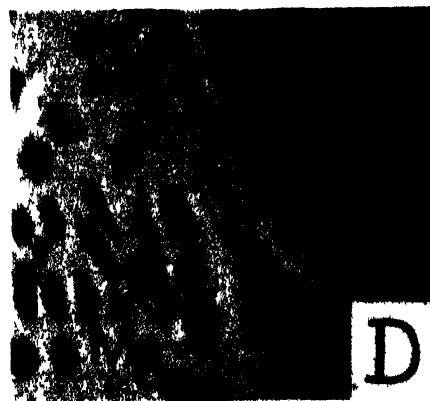
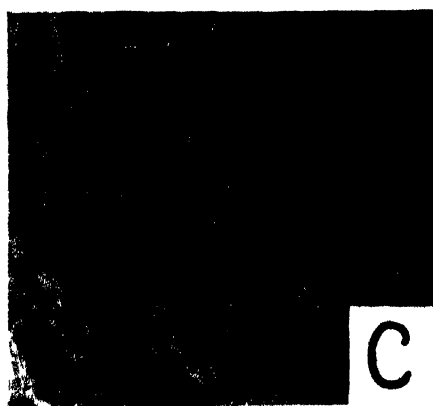
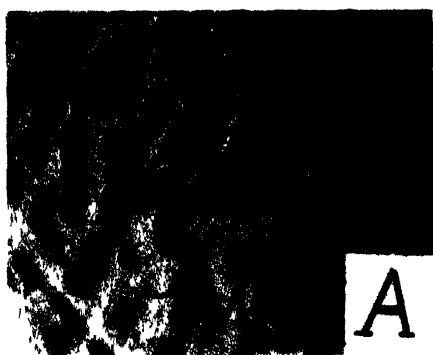
In the earliest stage the normal archesporium (M_C , Fig. 1-A) is found just below the nucellar epithelium. The somatic, aposporous cell (A_P) is distinguishable at the same time behind the archesporium near the chalaza.

* La Cour, L. A. *J. Roy. Microsc. Soc.* 51 : 119-126. 1931.

** In laboratory outline for cytology, Department of Botany, University of Toronto.

*** Hillary, B. B. *Bot. Gaz.* 10(2) : 276-300. 1939.

† Department of Botany and the School of Practical Science, University of Toronto.



Photomicrographs of longitudinal sections of young ovules in different embryonical stages. Figs. A, B, C, D, and E, Poa arctica. Fig. F, Poa alpina. Figs. A and C are from the same nucellus. See legend under Fig. 1.

Development of the normal type embryo sac from the normal archespore

The normal embryo sac develops from the fourth and innermost of four macrospores formed by two meiotic divisions from the normal archespore. The development is diagrammatically shown in Fig. 1. The normal archespore is first found just below the nucellar epithelium (Fig. 1-A and Plate I-C). A later stage showing the nucleus in prophase is shown in Plate I-B. The archespore occupies a large part of the young nucellus and, the nucleus especially, undergoes considerable growth before the meiotic division. Two daughter cells with the reduced chromosome number are formed (Fig. 1-B) and these give rise to four macrospores (Fig. 1-C and Plate I-D). The three outermost of these degenerate. (In Plate I-D the outer two have begun degeneration.) The fourth and innermost undergoes a period of growth and of vacuolization (sap-uptake) in preparation for the first embryo sac nuclear division. The fourth macrospore in Plate I-D has already increased in size (its nucleus is somewhat out of focus in the photograph). Not all the nuclear divisions in the embryo sac have been followed but young embryo

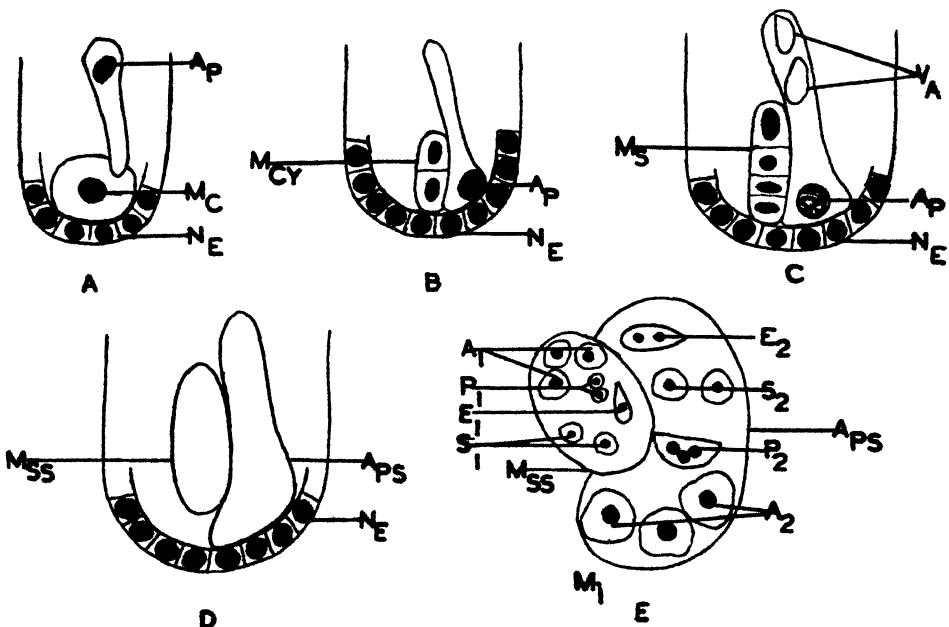


FIG. 1. Normal embryo sac development accompanied by apospory as found in *P. arctica* R. Br. from Greenland, diagrammatically illustrated. A_P , aposporous cell; A_{PS} , aposporous embryo sac; A , antipodals; E , egg cell; P , polar cell; S , synergids; M_{SS} , macrospore embryo sac; M_1 , micropyle; M_C , macrospore mother cell (normal archespore); M_B , macrospores; N_E , nucellar epithelium; M_{CY} , macrosporocytes; two archespore daughter cells; V_A , vacuoles. A. Normal archespore below nucellar epithelium, elongating aposporous cell behind it. B. Two archespore daughter cells. The aposporous cell has reached the micropyle and its nucleus has wandered out into the tip. C. Four macrospores. The nucleus of the aposporous cell in prophase before first embryo sac division. D. Two young twin embryo sacs. E. Two fully developed twin embryo sacs. Constructed lateral view of the twin embryo sacs photographed in Plate I-E.

sacs with four and six nuclei respectively have been observed and also several fully developed eight-celled embryo sacs. Although they are smaller than the twin (aposporous) embryo sac and somewhat crowded by it, each contains a uninucleate egg cell, two uninucleate unfused polar cells, three antipodals, and two synergids (Fig. 2-E and Plate I-E).

Development of a somatic aposporous cell into the second embryo sac

The earliest stage of development of the somatic aposporous cell is distinguishable at the same time as the earliest stage of the normal archesporium and is shown in Fig. 1-A and Plate I-A. A and C (Plate I) are photographs of sections of the same nucellus only 8 μ apart. (These are diagrammatically shown in Fig. 1.) It will be noticed that the aposporous cell that lies near the chalaza behind the archesporium first stretches in the direction of the micropyle (Fig. 1-A). When its tip reaches the nucellar epithelium it flattens out somewhat and the nucleus which has wandered from its original position reaches the cell tip at about the time that the archesporium has developed into two daughter cells (Fig. 1-B). The aposporous cell now undergoes further growth which involves a great sap-intake (see vacuoles, Fig. 1-C). These cannot be seen in the photograph (Plate I-D) but could be easily seen when the section was first mounted and stained.

Some increase in the size of the nucleus is evident and the aposporous cell is now ready for its first embryo sac division. The nuclear divisions of this embryo sac are not at present completely understood but they appear to be mitotic.

This embryo sac is seven-celled (Fig. 1-E). The three antipodals are large and two synergids are also present.

Competition of the two embryo sacs

The competition between the two embryo sacs for nutrition and space must mainly depend on the fact that the aposporous embryo sac, because of its origin near the chalaza (and thus the vascular system), has an immediate advantage. Factors influencing rate of growth of both embryo sacs must next be considered, that is, whether these are genetic or connected with nutrition and water-balance of the ovule as conditioned by these two factors for the whole plant.

It seems that the normal embryo sac survives surprisingly well and the author believes that the usual twin embryo sac combination is one normal and one aposporous. Death of either the normal or aposporous embryo must be expected in some ovules leaving the other embryo sac the full space.

DEVELOPMENT OF EMBRYOS AND ENDOSPERM

Parthenogenetic development of embryos

Parthenogenesis of the normal egg cell

Embryological evidence is not yet clear for *P. arctica* but the occurrence of weak twin and single seedlings, much less vigorous, smaller, and with much narrower leaves than the sister seedlings seems to point also to parthenogenetic

development of the normal egg. Twins, one haploid, one diploid are believed to be found in *P. alpigena*.^{*} The evidence for parthenogenesis of the normal egg in *P. pratensis* and *P. compressa* seems very convincing especially as interpreted from the careful observations of Miss Andersen (3)** and also from Tinney (11). Although none of the investigators who have shown the embryological evidence in their illustrations reports it*, Müntzing (8) shows that two *P. pratensis* plants that obviously are twins have 36 and 72 chromosomes respectively but he could not, at the time, give the embryological explanation.*

Parthenogenesis of the aposporous egg cell

That the aposporous egg cell develops parthenogenetically is indicated by the fact that it is multicellular before anthesis or pollination, and emasculated, unpollinated flowers dissected at harvest time can show development of embryos. Since there is no endosperm, however, there is no seed formation. Further, inhibited pollen tube growth in the stigma shows that fertilization is not the rule (5) and this, combined with the matroclinic appearance of all the plants raised from cross pollinations (5) shows that these originated from the aposporous ($2n$) egg cell. That the aposporous embryo develops by parthenogenesis in *P. pratensis* and *P. compressa* is evident from Miss Andersen's work (3) although she could not interpret it at the time. Tinney (11) states for *P. pratensis*, "the diploid egg develops into a proembryo by parthenogenesis. The development frequently begins before pollination."

Role of pollination in endosperm development (pseudogamy)

As already mentioned, the endosperm was not developed in emasculated, non-pollinated flowers although embryos were found. Pollination was thus found necessary for development of a complete seed in *P. arctica*, *P. alpigena*, and in *P. alpina* as well as in *P. pratensis* (5).

Aakerberg (1) reported that no seed was produced in *P. pratensis* in emasculated, unpollinated flowers and suggested pseudogamy as necessary for seed production.

Observations by the author of pollen germination and pollen tube growth from 2 to 36 hr. after pollination on the above *Poa* species showed that the ovary had increased in size at that time but the pollen tubes remained short. These facts combined with the embryological data made it evident that the germination of the pollen on the stigmas stimulated the development of the aposporous polar cell to an endosperm (pseudogamy).

Miss Andersen's data show clear evidence of this phenomenon in both *P. pratensis* and *P. compressa*, although she could not interpret this at the time. Her Plate 7-B shows two embryos (the "sexual" (?) and the aposporous ($2n$)) close to a large developing endosperm above which are the two nuclei of the fused but undeveloped polar cells of the normal embryo sac.

* See section on Reinterpretation of some other papers on *Poa*.

** See Plate 7-B and Plate 8-C in (3).

Tinney (11) suggests for *P. pratensis* that "since endosperm development was not observed to begin until after pollination, it may be that pollination or the growth of pollen tubes in stylar tissue is necessary for endosperm development and consequently for seed development. Pollen tubes have not been observed in the embryo sac." In Andersen's Plate 8-C (3), the scutellum of the larger, aposporous embryo lies pressed in against the endosperm whereas the smaller embryo does not reach the endosperm at all.

Tinney (11) shows in his Plate 3-II and Plate 4-B, two embryos almost imbedded in the large endosperm. In Plate 3-II the polar cell of the normal embryo sac lies undeveloped.* Tinney does not believe the normal embryo sac develops.

Andersen's Plate 7-B and Tinney's Plate 3-II and also Andersen's Plate 8-C and Tinney's Plate 4-B compare well.

DEVELOPMENT OF TWIN SEEDLINGS

Parthenogenetic development of the normal egg cell is believed by the author to accompany parthenogenesis of the aposporous egg cell.

Twin pairs of plants, one member showing matroclinic ($2n$) characters, the other showing aberrant characters have been obtained recently in numbers up to 5% in *P. alpigena* and *P. pratensis*.

*Twins, both of which show matroclinic ($2n$) characters, have been found in one half of one per cent of germinated seeds in *P. alpigena* and in *P. pratensis* and in larger proportions of *P. arctica* and *P. alpina*.

A paper dealing with these twin plants and their origin as well as the method used in raising them is being prepared.

The author believes that if both the "sexual" (n) and the aposporous ($2n$) embryos are in favourable positions in relation to the mass of the aposporous endosperm then this can nourish both of them to germination.

The scutellum of the *Poa* seed is a shield-like organ of absorption (3) through which the growing parts of the embryo receive their nutriment from the endosperm during germination. Andersen (3) shows stages of development of the embryos of *P. pratensis* and *P. compressa* and the extension of the scutellum. The author believes that only in the rare instances of actual fertilization by the pollen will the polar cells of the normal embryo sac develop into an endosperm. Fusion of the two cells may happen after pollination.

It seems evident that the aposporous embryo (and endosperm) give rise to the usual and most common plants, hence the matroclinic appearance of such great proportions of progeny (5, 12). Further, one cause of variation in progeny must be the fact that the "sexual" (n) embryo develops and produces a seedling because it is in a sufficiently favourable position in relation to the

* See section ~~on~~ Reinterpretation of some other papers on *Poa*.

aposporous endosperm to be nourished by it and also because of rare instances of fertilization. As they seem morphologically similar to the haploid member of twin seedlings many non-viable or weak seedlings encountered in greenhouse work may be from haploid embryos. There may be a slower or lesser scutellum development in this seed than in the aposporous one. If, of twin plants, one is diploid and one triploid as reported by Müntzing (7) then the possibility of their origin may be as follows: the diploid plant may have arisen from a fertilized normal egg cell and the triploid plant from a fertilized aposporous egg cell. Fertilization can occur in rare cases. Possibilities of development of cells other than egg cells into embryos (apogamety) will be considered in a later paper.

REINTERPRETATION OF SOME OTHER PAPERS ON *Poa* IN THE LIGHT OF THE PRESENT FINDINGS

The development of four macrospores from the normal archesporium was reported by Andersen (3) and Armstrong (4). A normal archesporium, but according to his illustration a very different form from the ones found by the author and others, was reported recently by Aakerberg (2). Tinney (11) reports "a single, elongated, very conspicuous macrospore mother cell with the nucleus located usually near the micropylar end, or in some instances near the chalazal end". This shows clearly that he has confused the aposporous cell with the normal archesporium cell. He believes three or four macrospores are formed but that they all die. Aamodt (12) believes "that there is no apparent reason why an occasional macrospore should be prevented from functioning and, if an embryo sac were formed, the haploid egg might either produce an embryo by parthenogenesis or develop into an embryo following fertilization."

Andersen (3) and Armstrong (4) believe that two macrospores can develop into embryo sacs. Aakerberg (2) and Tinney (11) are of the opinion that all macrospores die.

Andersen and Armstrong both confuse the nucleated tip of the aposporous cell with the "macrospore" near the micropyle that "sometimes" develops. Armstrong's Fig. 33 shows the aposporous cell cut in half.

Aakerberg (2) reports an aposporous cell but his illustration shows a type of undifferentiated cell found in numbers in the chalazal part of young nucelli before the aposporous cell shows up; he could not have seen the characteristic elongation of this cell.

Tinney (11) fully recognizes apospory (he finds approximately the diploid chromosome number in young embryo sacs). But he shows the fourth macrospore in the vacuolization stage and believes it to be "a somatic cell differentiated from the nucellus" and "destined to function as the initial cell of the embryo sac." He shows in Plate I-C the fourth macrospore with a large vacuole, and a smaller aposporous cell next to it that is just beginning to stretch. He believes these two cells to be the two twin embryo sac

primordia. In the same illustration he shows the fully developed aposporous cell which he believes is the normal archesporæ.

It is commonly agreed by all workers that polyembryony is very frequent, but the origin of the twin embryo sac has been the disputed point. In Table I the author has outlined the theories of the various workers and her own conclusions.

TABLE I
REPORTS OF VARIOUS AUTHORS CONCERNING THE OCCURRENCE AND ORIGIN OF EMBRYO SACS AND SEEDLINGS

Author	Twin embryo sacs			Twin plants			
	Occurrence	Origin		Occurrence	Origin		
		Normal	Aposporous		Haploid (reduced)	Diploid (unreduced)	Triploid
Andersen	Yes	Two	—	—	—	—	—
Armstrong	Yes	Two	—	—	—	—	—
Aakerberg	—	—	—	Yes	Reduced	Unreduced	—
Tinney	Yes	—	Two	—	—	—	—
Müntzing	—	—	—	Yes	Haploid (36)	Diploid (72)	Triploid
Author	Yes	One	One	Yes	Haploid	Diploid Both diploid (matroclinic)	—

Apomixis (parthenogenesis) and pseudogamy have been recognized in *Poa* since Müntzing (7) and Aakerberg (1) established knowledge of these facts, but it is only recently that Tinney (11) and the author have suggested new theories that explain the details of the mechanism of these phenomena.

Tables II and III show the early and new theories tabulated according to author and seniority and sequence of findings.

From the evidence given in this paper and an interpretation of the literature, the author feels that the twin embryo sacs so commonly found in the polyploid, polymorphous *Poa* species are, as a rule, developed one from a macrospore, the other from an aposporous cell.

It should be emphasized that clear evidence is found of both the development of the normal and the aposporous embryo sac especially in Andersen's, and also Armstrong's figures, and in descriptions and illustrations in Tinney's (11) work. The latter mentions in his paper "the smaller embryo sac" and the "unfused polar cells" which belong to it, also endosperm cells with four and five nuclei (here interpreted as belonging to aposporous embryo sac). He mentions twin embryo sacs where "there were five polar nuclei in one embryo sac and two in a companion sac."

To explain the aberrant types appearing in his progeny test of *P. pratensis*, Tinney and Aamodt (12) had to postulate the development of the occasional macrospore. There seems to be a difference between biotypes in *P. alpina*

TABLE II

APOMIXIS (PARTHENOGENESIS) AND PSEUDOGAMY AS REPORTED IN THE GENUS *Poa*

	Author					
	Müntzing 1932	Kiellander 1935	Aakerberg 1936	Flovik 1937	Tinney 1940	Present author 1940
	Species					
	<i>P. alpina</i> <i>P. pratensis</i>	<i>P. serotina</i>	<i>P. alpina</i> <i>P. pratensis</i>	<i>P. arctica</i> <i>P. alpigena</i>	<i>P. pratensis</i>	<i>P. arctica</i> <i>P. alpina</i> <i>P. alpigena</i> <i>P. pratensis</i>
Apomictic seed production reported on basis of:	Cytology and breeding experiments	Cytology	Breeding experiments	Cytology	—	Breeding experiments
Necessity of pollination for seed production and activation of embryo sac. Pseudogamy reported on basis of:	—	—	Pollination experiments	—	—	(a) Pollination experiments (b) Pollen tube growth studies

TABLE III

PARTHENOGENETIC DEVELOPMENT OF EMBRYOS AND ENDOSPERM

	Author	
	Tinney 1940	Present author 1940
	Species	
	<i>P. pratensis</i>	<i>P. arctica</i> <i>P. alpigena</i> <i>P. alpina</i> <i>P. pratensis</i>
I. Parthenogenesis of unreduced aposporous egg cell before pollination reported on basis of:	Embryological observations	(a) Matroclinic appearance of progeny (b) Embryological observations (<i>P. arctica</i>) (c) Embryos in unpollinated flowers (d) Interpretation of literature of <i>P. pratensis</i> and <i>P. compressa</i>
II. Parthenogenesis of normal egg cell reported on basis of:		(a) Twins in <i>P. alpigena</i> and <i>P. pratensis</i> (b) Interpretation of literature on <i>P. pratensis</i> and <i>P. compressa</i>
III. Endosperm development of aposporous polar cell by pseudogamy after pollination reported on basis of:	Embryological observation before and after pollination	(a) Observation of lack of endosperm in unpollinated flowers (b) Interpretation of literature on <i>P. pratensis</i> and <i>P. compressa</i>

and *P. pratensis* in "sexuality" as reported by Müntzing (8) and Aakerberg (2). This difference may be based on the fact that the normal egg cell develops more often in some strains than others.

It should be borne in mind that the ability of the haploid embryo to survive in *P. pratensis* and *P. compressa* may vary from one strain to another, and certain strains may therefore show a higher percentage of aberrants or higher percentage of twins than others.

Aakerberg (2) reports that 11.2% of the plants in an "apomictic" strain were twins and that 2.9% were twins in a "sexual" strain. The fact that there are twins at all in the "sexual" strain points to apospory being present here also, although Aakerberg claims not to have found aposporous embryo sac mother cells in this strain.

Plant breeding selection work with *P. pratensis* and *P. compressa* could be facilitated considerably if an initial embryological and seed germination investigation were made to determine the degree and type of twinning and thereby the number of aberrants to be expected.

Further work on the pairs of aberrant and matroclinic twin plants as well as on the pairs of matroclinic twin plants will be compiled for publication.

Acknowledgments

The author is greatly indebted to Dr. O. McConkey for suggesting the *Poa* problem and for suggesting the importation of seed from Greenland; to Magister M. P. Porsild, Director of the Danish Arctic Station, Disko Island, Greenland, for personally collecting and sending the *Poa* seed and for his valuable advice; to Dr. J. W. MacArthur for direction, advice, and never failing interest in the development of the problem.

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STERILITY IN POTATOES¹

BY T. J. ARNASON²

Abstract

Microspore tetrads were rarely formed in three male-sterile potato varieties studied. Dyads and triads were much more common. The failure of the second meiotic divisions appeared to be the main reason for the lack of tetrads. Many of the microspores aborted without enlarging, some did grow, however, and round off. In presumably mature anthers from open flowers, microspores were very variable in size and in the appearance of the nucleus and cytoplasm. Anther pores usually failed to open. The pollen-fertile varieties formed microspores that were almost entirely in tetrads. Mature anthers had roomy locules, open anther pores, and large numbers of uniform, sound-appearing pollen grains. They contained also some empty grains. About one-half the grains from one pollen-fertile line were empty. Abscission of buds and flowers is an important factor contributing to sterility or at least unfruitfulness in many potato varieties, including the three male-sterile ones reported in this paper. Following pollination with sound pollen, a few seeds have been obtained, though with some difficulty, from each of the male-sterile lines. Premature flower abscission rather than female sterility appears to be the main bar to seed production when sound pollen is applied.

Introduction

Breeding programs designed to discover the genetics of potato characters and those designed to produce superior new lines are often hampered by sterility. Many of the varieties most productive of good tubers ordinarily do not set seed. Selection for superior tubers may involve selection of non-fertile plants since in fertile plants more carbohydrate must be retained in the fruiting tops of the plants than in unfertile ones. With advances in knowledge of sterility in potatoes considerable saving of effort in breeding for ideal commercial types may be effected. That new varieties are needed is evidenced, for instance, by the fact that in Western Canada no early maturing, virus resistant, scab resistant, shallow eyed, well shaped potato of good cooking quality exists.

Each of the commercial varieties of potatoes has its own combination of characters, good and bad. The incorporation of the good characters of different clones in individual derivatives is difficult because of the sterility of most of the commercial varieties. It is possible that when the reasons for sterility are known, methods of overcoming it may be found in some cases at least.

A number of workers have investigated sterility in potatoes. It has been shown that failure to set seed may be attributed to bud abscission, meiotic irregularities in anthers, abortion of microspores, and abortion of embryo sacs. Stout and Clark (4) have supplied evidence to show that certain potato varieties may shed all their buds under certain conditions, form many flowers

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under other conditions. Sterility in which early abscission is the chief cause might be overcome by keeping plants cool when crosses were desired. The evidence (4) indicates that relatively low temperatures favour the retention of buds and flowers.

Failure of the meiotic divisions in anthers has been observed by a number of investigators (1, 2, and 6). According to Stow (5) meiotic failure is to be attributed to high temperatures. An experiment conducted by Ellison (1) failed to confirm this conclusion. Genetic as well as environmental factors must be taken into consideration since different varieties grown under similar conditions differ with respect to amount and length of flowering, regularity of meiotic divisions and pollen and seed production. Female sterility is much less marked than male sterility. Rees-Leonard (3) has reported, however, some evidence of embryo sac abortion in the variety Irish Cobbler.

Materials and Methods

Twenty lines of potatoes have been kept under observation for four seasons. Of these 15 were numbered seedling lines obtained from Dr. G. Rieman of the University of Wisconsin and five were commercial varieties kindly supplied by Dr. C. F. Patterson of the University of Saskatchewan. Three sterile or unfertile kinds were selected for special study. These were the United States Department of Agriculture selections 46000 and 44488 (Sebago) and the variety Early Ohio. Five lines were highly pollen-fertile. These were the U.S.D.A. selections 46422 and 45075 (Earlaine), Minnesota selections 11-1-2-1, 82-11, and 75-5. Observations were made on bud, flower, and fruit abscission, quality and quantity of pollen, and on meiotic divisions in anthers.

Pollen from ripe anthers was studied in aceto-carmin mounts. Meiotic divisions were studied in aceto-carmin smear preparations and in permanent sections. Material to be sectioned was fixed for 1 min. in Carnoy's fluid (3 parts absolute alcohol : 1 part glacial acetic acid) then in Karpechenko's modification of Navashin's fixative for 12-24 hr. The anthers were imbedded in Parlux which is superior to ordinary wax. The sections were cut at thicknesses of 12 to 16 μ and stained in Delafield's haematoxylin for 1 min. before passing through crystal violet and iodine solutions.

Bud Abscission and Length of Flowering Period

Failure of potatoes to set seed is not always owing to pollen or embryo sac sterility. The abscission of buds, flowers, and young fruits effectively prevents the formation, development, or ripening of seeds in many cases. The proportion of buds and flowers that drop off varies between varieties and also within varieties grown in different seasons or under different conditions.

In two consecutive seasons all the buds of Early Ohio plants dropped off before the flowers opened. In the third season after a particularly cold and wet June many flowers opened in the first week of July, but later buds, formed and developed in hot dry weather, all dropped off. The U.S.D.A. 46000

line behaved similarly except that a much larger number of buds were formed and a few of the buds developed into open flowers each season. Quite commonly there were 15 or more buds in a young cluster. Seldom did more than 10 of these reach the open flower stage, often the number was much lower. When flowers opened they had a tendency to persist for several days at least. Plants of the U.S.D.A. 44488 (Sebago) line formed fewer buds than did U.S.D.A. 46000. Most of the buds dropped off in each season but single open flowers here and there showed that the abscission mechanism did not cut off all buds before flowering. However, the open flowers almost invariably fell off within a few days.

Flowers of the U.S.D.A. 45075 (Earlaine) pollen-fertile variety were formed in abundance in all four seasons. Clusters of seven or more flowers were common. Even so, however, there was always some bud abscission. The length of the flowering period of this and other pollen-fertile varieties varied (Table I) from season to season indicating the importance of undetermined environmental factors. In some of the fertile varieties, buds were formed usually over a period of several weeks. The flowering period would undoubtedly be considerably longer for most varieties if there were not abscission of all buds during a part of the season.

TABLE I

LENGTH OF FLOWERING PERIOD OF SEVERAL VARIETIES OF POTATOES GROWN AT SASKATOON¹

Variety	1937	1938	1939	1940
U.S.D.A. 46000	July 24 – Aug. 19	July 5 – 12	July 7 – 15	June 28 – Aug. 5
U.S.D.A. 44488	July 25 – Aug. 19	July 16 – 29	July 2 – 19	June 21 – Aug. 5
U.S.D.A. 45075	June 23 – Aug. 17	July 5 – 14	June 29 – July 15	June 17 – July 22
Minn. 11-1-2-1	July 30 – Aug. 15	July 14 – 30	July 6 – Aug. 15	July 2 – 29
Minn. 82-11	July 15 – Aug. 19	July 5 – Aug. 2	July 2 – 15	June 24 – July 29
Minn. 75-5	July 1 – Aug. 5	July 12 – 30	July 5 – Aug. 15	June 28 – July 29
Irish Cobbler	Not recorded	? – July 23 – ?	June 29 – July 25	June 24 – July 15
Early Ohio	No flowers	No flowers	July 2 – 10	No flowers

¹ The pedigree numbers and names are those supplied with the original samples of tubers.

Mature Pollen

When ripe anthers of pollen-fertile potatoes are tapped sharply on a hard object, such as a thumb nail, easily seen dry yellow pollen is sifted through the subterminal pores and deposited. Although numerous trials were made, especially with the U.S.D.A. 46000 line, U.S.D.A. 46000 and Early Ohio anthers did not yield any pollen. In Table II are given the average percentages of sound-appearing grains in many pollen samples examined. In all varieties there was considerable variation in the proportion of good grains. Consequently the percentages given should be considered as no more than generally indicative of soundness. A point not brought out by the table is the fact that samples taken from any productive variety at different times in the same season show considerable differences.

TABLE II

NUMBER OF SOUND-APPEARING GRAINS IN POLLEN SAMPLES (AVERAGE PERCENTAGES)

Variety	1937	1938	1939	1940
U.S.D.A. 46000	No pollen	No pollen	No pollen	No pollen
U.S.D.A. 44488	13.3*	17.8*	No pollen	1.0*
U.S.D.A. 46422	61.5	55.7	41.9	39.0
U.S.D.A. 45075	16	72.6	60.9	58.8
Minn. 11-1-2-1	84.7	93.4	94.0	79.0
Minn. 82-11	64.6	89.8	56.9	75.8
Minn. 75-5	53.0	76.8	82.5	60.0
Early Ohio	No flowers	No flowers	No pollen	No flowers

* Pollen very scanty.

NOTE: The soundness of the pollen of U.S.D.A. 45075, Minn. 11-1-2-1, 82-11, and 75-5 has also been proved by successful crosses.

Meiotic Divisions

A small proportion of cells of both pollen-fertile and pollen-sterile varieties showed slight irregularities in the first meiotic division. In both, however, bivalents at metaphase and two compact chromosome groups at late anaphase were the rule. It is mainly at the conclusion of this division that the pollen-sterile lines begin to show noticeable peculiarities in nuclear behaviour.

In the pollen-fertile U.S.D.A. 46422 and 45075, the second division follows the first rather closely. The two spindles of the second division are parallel in some cells, in others at right angles to each other. Counts of cells of U.S.D.A. 46422 gave 51 with spindles parallel or nearly so, 27 with the spindles at right angles. In these varieties wall formation begins shortly after the

second nuclear division has taken place. Spore tetrads occur in abundance. Malformed or abnormal cells are rare or absent.

Deviations from the "normal" development of the pollen-fertile lines become marked after the first division in all three of the pollen-sterile lines examined. The cause of the breakdown at this stage has not been determined. Since some differences between the cells of the sterile varieties were observed they will be described separately.

U.S.D.A. 44488

Twenty-four chromosomes were counted in several polar views of first division anaphases. As in the fertile material, no walls were formed at the end of this division. The two nuclei moved to near the centre of the cell, enlarging considerably before the nuclear membranes disappeared. At the second division metaphases, the chromosomes of the two nuclei sometimes were grouped together on a single plate (Fig. 12). Frequently two metaphase plates were visible however, but the orientation of these was often highly irregular, e.g., the two spindles converged on one side or the spindles were crossed so that at anaphases some chromosomes were apparently stranded in the mid-region (Fig. 14). In late anaphases the chromosomes were in two, three, or four groups. Single chromosomes sometimes failed to be incorporated in the telophase nuclei. The number of normal appearing spore tetrads formed was small. A count of the number of cells in 100 cells or groups derived from single pollen mother cells gave the following results:

4 cells	(tetrads) approximately equal in size	7 groups
4 cells	markedly unequal	11 groups
3 cells	unequal in size	41 groups
2 cells	some equal, some not equal	40 groups
1 cell		1
		<hr/> 100

In the dyads and triads densely staining granules (Figs. 21, 22) were visible in about 25% of the cells. These were probably chromosomes that had failed to be included in the nuclei. Lobed nuclei, formed by fusion of more or less distinct chromosome groups, were common.

U.S.D.A. 46000

The first meiotic division was slightly more irregular in this than in other lines. Even so, the majority of late anaphase figures showed two compact chromosome groups. A count of 84 cells gave 80 with no visible irregularity in late anaphases, three cells with one or two lagging chromosomes, and one cell with a thin chromatin strand connecting the two chromosome groups. On the whole there was little visible irregularity until after the first division telophases. Following the telophase stages the nuclei moved toward the centre of the cell becoming somewhat pycnotic and shrinking in size. Few second division figures could be found, probably because they failed to occur.

round, to be expected if nuclear fusion preceded the formation of the plate. More often the plate appeared as two overlapping circles, an appearance to be expected if the nuclei were very close together or only slightly fused at the time of the disappearance of the nuclear membranes (Fig. 10). In side view the second division plate was narrower and had smoother margins than first division plates. The individual chromosomes were smaller and, of course, more numerous. Some of the second division plates as observed in side view were curved or bent in L, V, or T shape. These had tripolar spindles.

In a few cells two distinct second division metaphase plates were seen (Fig. 15). At later stages very few tetrads of spores occurred. Counts were not made but in the material examined (several thousand cells from many anthers collected at different times) probably less than 1% of pollen mother cells formed tetrads. It is not certain that a second division always takes place. In any case by the time wall formation is completed dyads are most abundant, triads are rare, and tetrads very rare.

Some of the dyad microspores grew in size, rounded off, and separated. However, sections of open flowers showed that up to that time most of the dyads had remained small and many were flattened.

Microspores

Sections of anthers of U.S.D.A. 44488 containing young microspores were examined. Many rounded microspores of varying sizes were seen (Fig. 22). Most of the dyad cells apparently had rounded off and separated. The nuclei of these spores were far from uniform in appearance. The two nuclei found in some of the young microspores (Fig. 4) were doubtless derived from the second meiotic division, i.e., a division of a spore nucleus to form a tube and generative nucleus was not responsible.

In 100 young microspores of U.S.D.A. 44488 the nuclei appeared as follows:

Nuclei	Number of spores
Single round nucleus	23
Round nucleus and one or more micronuclei	2
Single lobed nucleus	61
Lobed nucleus and one or more micronuclei	4
Two or more nuclei nearly equal in size	10
Total	100

At the young microspore stage few dyads were visible. The young microspores assumed a spherical or almost spherical form and were present in rather a diversity of sizes. Nuclei were variable in size and shape. Lobed nuclei and micronuclei were not uncommon.

Sections of anthers of open flowers showed some normal appearing large pollen grains. The average diameter of 50 such grains was calculated to be $25\ \mu$ as compared to an average diameter of $28.7\ \mu$ in one pollen-fertile line and $26.2\ \mu$ in another. The total number of spores present in the locules was less than in fertile lines judging by the numbers present in the sections. A count of 50 sound-appearing grains to 82 empty was made in one longitudinal section. Some greatly enlarged dyads were seen. The microspores were probably formed mainly from dyads. No test of germinability has been made but it is likely that it was very low.

The locules of this variety are large and roomy. Remains of the tapetum form a very thin layer along the locule edges. Of the other varieties tested only the pollen-fertile lines had large locules.

In the Early Ohio variety the locules were small and usually contained a large quantity of disorganized tapetal material along with spores, many of which (about one-half) were empty; many of these were flattened. Of the remainder, dyad groups having a diameter of 15 to $21\ \mu$ were most common but there were also sound appearing pollen grains here and there. These were mostly about $21\ \mu$ in diameter.

In the U.S.D.A. 46000 line the locules, as in Early Ohio, were small at the open flower stage and contained considerable quantities of broken down tapetum. At this stage many small dyads were found. Microspores that occurred singly were almost all small and empty. Hundreds of anthers were examined. It is probably safe to say that in them less than 1% of the grains were sound.

Sectioned anthers of pollen-fertile lines at young free microspore stages showed, in general, spores uniform in size, each containing a single nearly spherical nucleus. Spore abortion (usually less and in some lines much less than 50%) frequently occurred late, after the spores had reached normal pollen grain size. In sections of nearly mature anthers of the pollen-fertile Minn. 82-11, 55% of the pollen grains were empty. Most of the empty grains were as large, or almost as large, as the sound appearing grains. Anthers of corresponding age from the sterile forms always contained spores in a great variety of sizes, aborting spores ranging from extremely tiny to larger than "normal" size. The sections of Minn. 82-11 revealed another peculiarity,—in some parts of the locules all the spores had aborted, in other "pockets" nearly all the grains were sound. This suggests that the abortion of microspores to the extent of about one-half in this variety may be due to slight structural or physiological defects in parts of the anthers rather than to genetic factors segregating in the meiotic divisions.

Seed Production and Seedlings

All three of the male-sterile lines proved to be capable of forming seeds

of U.S.D.A. 44488 were pollinated without success until 1940 when two fruits were matured following pollination with Minn. 75-5. Each fruit contained many seeds indicating that embryo sac abortion was not extensive. U.S.D.A. 46000 plants have formed a few fruits. As the number of seeds in fruits was always small, there may be some female sterility in this line. The pollen parents in successful crosses involving this line were the excellent pollen producers Minn. 11-1-2-1 and 75-5. Premature abscission of flowers is probably the chief obstacle to obtaining seeds in all three varieties.

Ten seedlings of U.S.D.A. 46000 \times Minn. 11-1-2-1 were grown for two consecutive years. Tests of pollen production and soundness were made. All the plants bloomed. Only three failed to yield any pollen when the ripe anthers were tapped. Three produced abundant pollen. One plant produced as high a percentage of sound pollen as the male parent (about 90%). One produced fruits following open pollination. It is probable that at least four out of 10 F_1 plants produce sufficient viable pollen to be used successfully in back-crosses to the male-sterile parent variety. Such a procedure may be desirable when the male-sterile variety is commercially valuable but has one or a few undesirable features not found in the pollen parent of the original cross.

Discussion and Conclusions

The formation of viable functioning potato pollen may be prevented by bud abscission, meiotic failure, or abortion of spores after tetrad formation. The importance of the first of these was recognized by Stout and Clark (4) who recognized also the importance of environmental factors, especially temperature, in influencing this abscission. Numerous reports on meiotic failure have appeared (1, 2, 5, 6). These have shown that at least in some varieties univalents are present at metaphase of the first meiotic division and that chromosome distribution to the two poles is not always equal. Monads may form by complete failure of the first division to occur or by fusion of chromosome groups after partial or complete separation. Dyads may result from complete failure of the second division, from fusion of the two second division spindles, and possibly in other ways. The observations made on the first meiotic division in connection with the present work indicated that failure of the first meiotic division was not conspicuous. Most of the failure observed became evident at the end of the first division or later. There probably are cases, however, in which failure of the second division is due to irregularities in the first.

In the male-sterile varieties studied the second division is considerably delayed in comparison to varieties that normally form tetrads. Possibly the first division is also delayed (1) although anther measurements of sterile and fertile lines failed to reveal any consistent size differences up to anaphase stages. The second division is perhaps omitted entirely in some cells. In

microspore tetrads at the conclusion of the meiotic divisions. In all of these lines the percentage of sound pollen grains fluctuates rather widely. If spore lethals were the sole cause of spore abortion little fluctuation should occur. Factors other than spore lethals must frequently operate to increase the proportion of aborted spores in such lines.

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Abstract

Using prepared samples of seed, in which impurities were represented by stained seed of the same kind, repeated samplings from a restricted bulk were made by the method being studied. After each sample was drawn, the number of stained seeds in it was recorded and it was then put back in the bulk. The data, being in the form of numbers in a unit weight, were first compared with the corresponding Poisson distribution, which, however, was found inapplicable. Working from first principles, an expression was found that was believed to suit the conditions of the experiment and that proved to be a special case of the binomial distribution, not previously applied, it is believed, to studies of this sort.

Statistical tests showed an excellent fit between observed results and the values expected according to the new expression, which thus provides a measure of the variability to be expected in drawing subsamples from a restricted bulk and at the same time provides the required basis for comparison of the seed sampling methods under study. It shows that less variation is to be expected between duplicate tests of a submitted sample than was previously thought to be the case.

Introduction

In the control of a commodity such as seed, which is notoriously difficult to sample satisfactorily, the question of methods of sampling assumes a prime importance. This is true not only of sampling from the bulk but also of the drawing of a satisfactory working sample from a sample submitted to the laboratory for test, which this forms the subject of this paper. The procedure adopted in the study was to compare the results of a large number of experiments by any particular sampling method with what would be expected on statistical grounds.

The laboratories referred to are those of the Laboratory Service, Plant Products Division, Dominion Department of Agriculture, Canada.

Materials and Methods

Briefly, the procedure followed was to prepare samples of seed in which impurities were represented by stained seed of the same kind; from these samples, which represent restricted bulks, repeated subsamplings were made by the method being studied. During the summer of 1939, samples of the following crop plants were distributed to various laboratories for studies as indicated:

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Contribution No. 656, from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa. Project S.S. B. 18.02.

² *Botanist (Physiology).*

Seed Research Laboratory, Ottawa	Boerner
Sweet clover	
Two 40-gm. samples each containing 80 stained seeds to:	
Sackville	Wright
Montreal	Wright
Ottawa	Wright
Seed Research Laboratory, Ottawa	Leggatt (2)
Winnipeg	Wright
Saskatoon	Wright
Vancouver	Wright

** The method referred to consists of mixing the submitted sample by pouring it back and forth between two large containers. The working sample is obtained by pouring the required weight on to the pan of a scale.*

The method of obtaining experimental data was the same for both cereals and clover. In the cereals, 32 of the stained seeds supplied were to be added to 4 lb. of seed of the same kind. This constituted the "submitted" sample corresponding to the 40-gm. samples of sweet clover.

After an initial mixing the submitted samples were to be repeatedly divided down by the mixer (or method) in question; samples were taken from alternate spouts, until four cuts had been made, providing a working sample 1/16 as large as the submitted sample. This working sample was spread on the analysis table, the number of stained seeds recorded, and then it was returned to restore the original submitted sample. This process was repeated, but without the initial mixing, until 1000 trials had been made. The data have been summarized and are given in Tables I, II, and III.

Theoretical Considerations

Previously, when considering data of this type, i.e., numbers of impurities per unit weight, it has been assumed that the Poisson distribution has been the proper criterion for comparison of the observational data. It has been noted on more than one occasion, however, in similar studies (1) where observed data which followed the binomial distribution were compared with the expected values, that there is a tendency towards a narrowing of the distribution curve, i.e., more values were observed near the mean and fewer towards the extremes than were expected. This tendency was ascribed to the probable effect of the restricted size of the bulk sample. Preliminary studies of the present data strongly indicated a similar tendency, so much so that it was felt that the matter should be studied further.

It is implicit for the validity of the Poisson distribution that the sample be drawn from an indefinitely large bulk; thus there is no restriction on the possible

For the data used in this study, however, the bulk from which the working sample was drawn is very far from being indefinitely larger than the latter. It is, in fact, only 16 times as large, the subsample being the result of four successive divisions-in-two, or dichotomies, D .

In the bulk or submitted sample from which the subsample was drawn the number of impurities is sharply limited. Since the bulk sample at the first dichotomy, D_1 , is divided into two equal parts, the chance of any given seed falling into one or other of the halves is $\frac{1}{2}$, or $p = .5$. At the second, D_2 , the chance of its falling into any one of the four possible subsamples is $\frac{1}{4}$ or $\frac{1}{2^2} = \frac{1}{2^d}$, where d is the order of dichotomy. Similarly, at D_3 , $p' = \frac{1}{8} = \frac{1}{2^3} = \frac{1}{2^d}$; and so on for any number of dichotomies, $p' = \frac{1}{2^d}$.

Since $q' = 1 - p'$, we have $q' = 1 - \frac{1}{2^d} = \frac{2^d - 1}{2^d}$ and since $m = n'p'$ we have $m = \frac{n'}{2^d}$ and also $n' = 2^d m$ which, of course, is the number of impurities in the bulk sample.

The distribution, then, is given by the expansion of $(p' + q')^{n'}$ where the symbols have the above meanings. This is an expression of the ordinary binomial form which has a variance, $V = n'p'q' = n' \cdot \frac{1}{2^d} \cdot \frac{(2^d - 1)}{2^d}$.

Now, as the number of dichotomies becomes very great, the expression $\frac{(2^d - 1)}{2^d} \rightarrow 1$ and n' becomes indefinitely large in comparison with m , while p' becomes indefinitely small. Thus this distribution merges with the Poisson distribution when the working subsample becomes an indefinitely small fraction of the bulk sample, as would be expected.

In the following tabulation certain statistics are collected for reference and comparison. The broken infinity symbol \propto is used to indicate an indefinitely large quantity, not however reaching infinity.

Distribution	Expansion of:	Variance	m	p	q	n
Binomial	$(p + q)^n$	$n p q$	$n p$	<1	$1 - p$	Any value
Poisson	$(p + q)^n$	np	np	$\rightarrow 0$	$\rightarrow 1$	\propto
Binomial derived by dichotomy	$(p' + q')^{n'}$	$\frac{n'}{2^d} \cdot \frac{(2^d - 1)}{2^d}$	$\frac{n'}{2^d}$	$\frac{1}{2^d}$	$\frac{2^d - 1}{2^d}$	$n' = \text{any value}$

Note: d = the number of dichotomies.

than the Poisson curve and explains the anomalies previously referred to. It is of practical significance in that it provides a measure of the variability to be expected in drawing subsamples from a restricted bulk and shows that less variation is to be expected between duplicate tests or analyses of a submitted sample than was previously believed to be the case. It also gives the proper criterion for making such comparisons as form the subject of this paper.

The distribution curve which is discussed above and which is obtained as the result of drawing samples from a comparatively small bulk or submitted sample is not restricted to the case where there is a succession of even dichotomies. It will be noted that the value $p' = \frac{1}{2^d}$ is simply the fraction of the submitted sample constituted by the working sample.

Let w = size of working sample

s = size of submitted sample

then

$$p' = \frac{w}{s}$$

$$q' = 1 - \frac{w}{s} = \frac{s - w}{s}$$

$$\therefore m = n'p' = \frac{n'w}{s}$$

$$V = \frac{n'w}{s} \cdot \frac{s - w}{s}$$

These symbols may be used when the working sample is any fraction, not necessarily an even submultiple, of the submitted sample.

Experimental Results

Sweet Clover

In Table I are presented the combined results obtained by the seven laboratories which analysed the sweet clover samples.

It will be seen that the "dichotomous" binomial distribution fitted the observed distribution very well but that the Poisson did not do so. This confirms the validity of the new theory as a basis for the comparison of results from restricted bulks and at the same time shows that the sampling methods used were, on the whole, satisfactory.

Of the seven stations taking part, six used the Wright sampler and one used the Leggatt sampler. In Table II the results of the χ^2 test are given to enable comparison of the two types of sampler and of the two theoretical distributions to be made.

Number stained seeds found	Number of analyses giving or expected to give numbers of stained seeds listed in Column 1		
	Observed	Poisson	"Dichotomous" binomial
0	46	50	43
1	212	246	225
2	560	608	585
3	1004	1002	1000
4	1265	1240	1267
5	1315	1228	1268
6	1051	1013	1043
7	725	716	726
8	447	443	436
9	220	244	229
10	86	121	107
11	37	54	45
12	18	22	17
13	12	9	6
14	2	3	2
15	-	1	1
TOTAL	7000	7000	7000
Mean	4.94	4.95*	4.94
χ^2		35.695	12.863
n		12	12
P		< .01	.39

* Average of distributions for $m = 4.9$ and $m = 5.0$.

TABLE II
COMPARISON OF RESULTS FROM WRIGHT AND LEGGATT SAMPLERS*

Sampler	Number tests	Compared with:	χ^2	n	P
Wright (Mean 4.94)	6000	Poisson	37.572	10	< .01
		"Dichotomous" binomial	16.257	10	.14
Leggatt (Mean 4.95)	1000	Poisson	12.317	10	.35
		"Dichotomous" binomial	11.379	10	.42

* In order to enable direct comparisons between the values of χ^2 to be made, the tests were brought to the same number of degrees of freedom by grouping certain values. This has brought about some improvement in the values of P which, therefore, cannot directly be compared with those in Table I.

In the following comparisons, where

P_1 = the probability that the greater value of χ^2 (χ^2_1) will be exceeded,

P_2 = the probability that the lesser value of χ^2 (χ^2_2) will be exceeded,

$1 - P_2$ = the probability that χ^2_2 will not be exceeded,

the difference in the value of χ^2 is highly significant, but for the Leggatt sampler there appear to be too few tests to demonstrate any difference.

Wheat and Oats

In Table III are presented the combined results obtained by the three laboratories which analysed the wheat and oat samples. These results can be combined because the quantities involved were the same for both species. They include data obtained both by the Boerner sampler and the "pouring" method.

TABLE III

RESULTS OF 4800 ANALYSES OF $\frac{1}{4}$ LB. SAMPLES REPEATEDLY DRAWN FROM BULK SAMPLES OF 4 LB. OF WHEAT OR OATS CONTAINING 32 STAINED WHEAT OR OAT SEEDS RESPECTIVELY, COMPARED WITH CORRESPONDING POISSON AND "DICHOTOMOUS" BINOMIAL DISTRIBUTIONS

Number stained seeds found	Number of analyses giving or expected to give numbers of stained seeds listed in Column 1		
	Observed	Poisson	"Dichotomous" binomial
0	579	637	609
1	1329	1286	1298
2	1330	1299	1342
3	881	875	894
4	450	442	432
5	166	179	161
6	51	60	48
7	12	17	12
8	2	4	3
9 or more	—	1	1
Total	4800	4800	4800
Mean	2.02	2.02	2.00
χ^2		12.848	3.875
n		6	6
P		<.05	.70

It will be noted here again, that the Poisson distribution differs significantly from the observed results but that the "dichotomous" binomial gives an excellent fit.

Using the "dichotomous" binomial distribution as the basis of comparison, if the results of the "pouring" method (with 2000 values) and the Boerner method (with 2800 values) are examined separately, it is found that $P = .18$

rest of the values (two or more) provide an excellent fit with expectation. It is probable that the pouring method would not be reliable for very low rates of occurrence of the impurity.

Acknowledgment

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CHANGES IN THE PHYSICAL PROPERTIES OF GLUTEN WITH AGING OF FLOUR¹

BY J. D. McCAIG² AND A. G. McCALLA²

Abstract

The physical properties of gluten, as determined by the hydration of the gluten between pH 4 and 7, are deleteriously affected by aging of flour. Similar effects are obtained by adding linolic acid, whereas some of the effects of aging can be removed by extraction of the flour with ether. The original quality of the gluten is not restored by such extraction. Gluten from aged deteriorated flour swells enormously in 0.1 *N* acetic acid; such hydration is not an indication of good quality but rather of resistance to dispersion. Some freshly milled flours produce gluten possessing the physical characteristics of gluten from aged deteriorated flour.

It is concluded that the quality of gluten depends to a considerable extent on the nature of the adsorbed lipoids. Many of the characteristics of gluten are determined by relatively insoluble (unidentified) lipid substances, whereas the absence of such lipoids and the formation of fatty acids during aging are deleterious to gluten quality.

Introduction

For the past seven years studies concerned with the effects of aging on the quality of wheat and flour have been carried on in this laboratory. Some of the earlier results have been published (1, 19), and have shown that the changes in quality of the flour with aging are intimately associated with changes in the physical properties of the gluten. This has, of course, been demonstrated by other workers (12, 22). Although gluten has been the subject of many investigations, the existing knowledge was inadequate to afford an interpretation of many of the results which we had obtained, so an intensive study of this substance was undertaken.

The extent of the work done with gluten is indicated in the Bibliography of Baking Quality Tests (11) published in 1934. This summary lists nearly 200 references to work in which gluten, directly or indirectly, was the subject of investigation. Most of these studies, particularly those of more recent years, were concerned with finding a simple, rapid test, to be carried out on flour or gluten, that would replace the baking test in evaluating the quality of wheat. Such a test has not been found, but our knowledge of gluten has been considerably extended. Only a few papers dealing with the properties of gluten can be considered here.

The view that gluten is made up of two proteins, glutenin and gliadin, and derives its physical properties from the properties of these two proteins (16, 23), is no longer widely held. The work of Sorensen (20) has shown that most

soluble proteins are not made up of individual chemical compounds but rather of what he termed component systems, in which the various components are held together more or less firmly by secondary or residual valencies. The individuality of glutenin and of gliadin has been widely questioned (3, 14, 21), and it seems likely that Sorensen's concept may be extended to include the protein of gluten. Sorensen also discussed the importance of lipoids in determining the physical properties of the serum proteins, and it has been shown that these substances play an important part in determining the physical properties of gluten (12, 19, 22).

Thirty years ago, Wood and Hardy (23) discussed the amphoteric nature of gluten protein and the conclusions they reached are, as far as they went, acceptable today with little modification, although their conclusions regarding the factors determining the physical properties of gluten are not. More recent studies (4, 5, 8, 9, 10, 15, 18) have added considerably to the views expressed by Wood and Hardy. Gortner and his co-workers (9, 10, 18) showed that the properties of gluten were not determined by the acid-salt balance in the flour or the gluten, but that there were inherent differences in the properties of gluten from different wheats regardless of the amounts or kinds of acids and salts present. These conclusions were reached as a result of studies involving the swelling of gluten in acid-salt solutions (9) and of viscosity studies (10, 18). The latter were considered the more satisfactory. It was concluded that weak gluten had a lower rate of hydration than strong gluten and that it changed from a gel to a sol at a lower degree of hydration, that is, it had a lower maximum hydration capacity. Newton and Cook (15) studied the bound-water of flour suspensions and concluded that hydration rates and capacities offered an inadequate explanation of the differences in properties of strong and weak gluten. They suggest that structure as well as hydration of gluten must be considered. Larmour and Sallans (13) obtained high correlation coefficients between viscosity of flour suspensions and loaf volume (bromate formula) with a series of Marquis wheat samples varying in protein content from 8.2 to 18.3%. Had these investigators been working with samples of several varieties and a narrower range of protein content, it seems certain that the significance of the correlations between viscosity and loaf volume would have been much lower.

The same principles that underlie the methods already mentioned (9, 10, 18, 23) also underlie the patented processes of Berliner and Koopman (2) and Ruemele (17). The former process utilizes the variability of the swelling of gluteins in weak acid to classify flours as weak or strong, whereas the latter uses variability in the viscosity of gluten dispersions of two concentrations in *N*/50 lactic acid. A full discussion of these methods cannot be undertaken here but other workers have not been able to substantiate all the claims made by the originators (8), although there are certainly measurable differences in different gluteins.

of hydrogen ion concentration. While it cannot be considered that his gliadin and glutenin were definite individual compounds, the importance of the work is hardly affected by this fact. He found (5) that the properties of mixtures were not the sum of the properties of the two components when the protein sols (in 0.001 *N* sodium hydroxide plus phosphate buffers) were between the pH values of 5.3 and 6.6, which he determined as the isoelectric points of his protein preparations. Between these two pH values one preparation was electronegative and one electropositive; mixing the two tended to neutralize the charges. The mixtures showed the greatest turbidity at different pH levels depending on the proportion of the two preparations used. It was concluded that there was an interaction between the two preparations in the pH range between the isoelectric points and that complete separation of the components could not be expected within this range.

If gluten as a whole is considered as a component system (20), then Bungenberg de Jong's preparations must be regarded as two main groups of components and the isoelectric point of each as the point at which the negative charges of some of the components just balance the positive charges of the remainder. If his two preparations were recombined in the proportions originally occurring in gluten, the isoelectric point (if it can be termed that) would be the point of balance between total positive and total negative charges. This point Bungenberg de Jong believes to be at pH 6.0 or 6.1.

In a previous paper (19) it was reported that the water absorbing capacity of glutens from deteriorated flour was lower than that of glutens from flour of good quality, between the pH values of 4 and 7.5. There also appeared to be a difference in behaviour of the two types of gluten as the pH changed from 6.8 to 7.5. At the time no explanation for this difference was offered but the studies have been continued and form the starting point for the work reported in this paper.

Material

The flour quality data that are pertinent to the present study are given in Table I. Gluten was washed by the method of Dill and Alsberg (6); acidity was determined by the Greek method (7), using tincture of curcuma as indicator. Since a discussion of particular comparisons is made throughout the paper, these data are not elaborated here. All flours except No. 11 were experimentally milled long patents, and were unbleached. The flours which were stored in the laboratory were kept in sealed containers at a moisture content of approximately 10 to 12%. Since this was not a study of storage effects, details of storage conditions are omitted.

A supply of the wheat from which No. 6 was milled in the spring of 1937 was stored in a cool bin and samples remilled in the spring of 1938 and 1939. Flour from the first milling was stored in a sealed container for two years and then restudied. Another lot of the same flour was stored for one month at

of moulds and bacteria, the interior of the desiccator was rinsed with toluene. A small container of toluene was also kept inside. At the end of the month this flour showed all the characteristics of an aged, deteriorated flour and was studied in comparison with the original.

TABLE I
DATA PERTAINING TO FLOURS USED IN STUDY

Flour No.	Variety	Time from milling, months	Protein in flour, %	Acidity, %*	Wet gluten, %**	Quality of wet gluten	Dry gluten, %**	Loaf volume cc.***
Main series								
1	Garnet	30	9.7	0.83	33.6	Very poor	11.7	340
2	Garnet	6	8.9	0.83	20.5	Very poor	8.0	480
3	Mixed	54	13.8	0.75	41.2	Very poor	16.0	430
4	Garnet	18	10.9	0.64	30.8	Poor	11.8	435
5	Garnet	6	13.5	0.55	41.6	Fairly good	14.6	668
6	Reward	2	15.0	0.27	55.0	Excellent	18.4	985
11	Soft wheat†	—	6.9	0.29	18.9	Soft	6.4	410
Special series								
7	Garnet	6	8.9	0.65	25.2	Poor	9.6	439
8	Garnet	6	13.4	0.49	40.7	Fairly good	14.8	657
9	Red Bobs	6	8.5	0.31	25.2	Fairly good	9.2	564
10	Red Bobs	6	14.3	0.43	47.0	Good	16.2	874

* Expressed as percentage C_{18} fatty acids.

** Obtained at pH 6.8.

*** Malt-phosphate-bromate formula.

† A commercial cake flour.

Results

Preliminary Experiments

The preliminary experiments involved a study of the effect of the pH of the washing solution on the water content of gluten and of the swelling in 0.1 *N* acetic acid of gluten from a wide variety of flours. Gluten balls were washed from sufficient flour to yield approximately 2.5 gm. of wet gluten. The washing solutions contained 0.1% phosphate buffer varying from pH 3.7 to 8.5. The gluten was dried in a vacuum oven at 98° C. for 24 hr. The experiments on the swelling of gluten in 0.1 *N* acetic acid were based on the method of Gortner and Doherty (9). Individual gluten balls washed at pH 6.8 instead of cut discs were used. Preliminary tests showed that gluten from deteriorated flour had enormous hydration capacity in acid and the use of more dilute or weaker acids resulted in prolonged swelling times. For this reason 0.1 *N* acetic acid was used.

The results of the experiments in which gluten was washed out using solutions of pH 3.7 to 8.5 are expressed in terms of water content of the wet

Two main conclusions emerge from this experiment. Both the hydration capacity of glutes from the different flours and the pH at which the gluten exhibits a minimum hydration, decrease with the quality of the gluten (see Table I for gluten quality notes). The gluten from soft wheat flour (No. 11) had the highest water-holding capacity of any of the glutes, and was very soft and extensible, but lacked elasticity. The gluten from flour No. 6, typical of that obtained from high grade, strong, hard, red spring wheat, possessed excellent elasticity, and was also firm and extensible regardless of the pH of the washing solution. The glutes from Flours 1, 2, and 3 were coarse, open, and very short. Those from Nos. 4 and 5 were intermediate in quality characteristics, No. 5 being decidedly better than No. 4. The "weakness" of flour No. 11 was very different from the "weakness" of Flours 1, 2, and 3, since the physical nature of the gluten was entirely different.

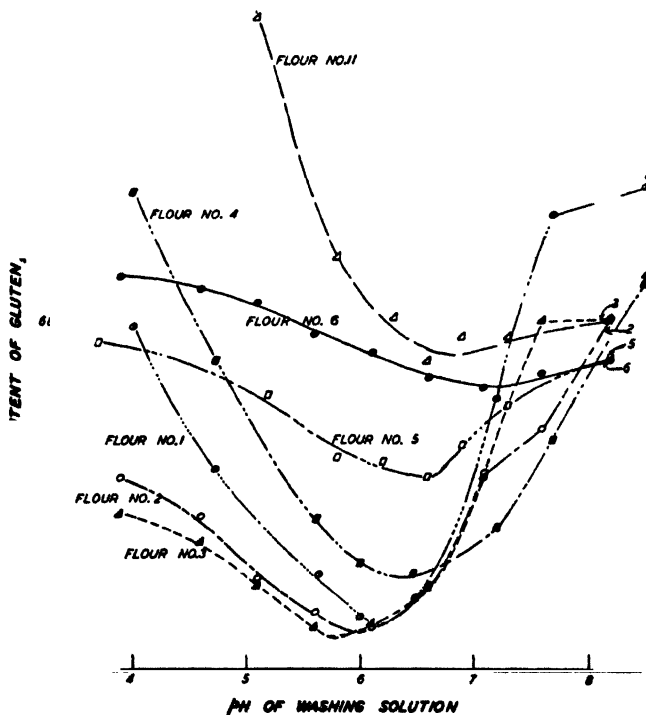


FIG. 1. The effect of pH on the hydration of gluten.

The results of the gluten swelling experiments expressed as increases in percentage water in the gluten are presented in Fig. 2. These results present a completely different picture, since the poor quality glutes have much the higher hydration capacity in 0.1 *N* acetic acid. These poor quality glutes did not disperse in 2.5 hr., whereas the gluten from flour No. 6 dispersed in

less than two hr. At 2.5 hr., the swollen gluten from flour No. 2 contained only 9% dry matter, but still formed a coherent mass which could be handled. In contrast to this, the gluten from flour No. 6 dispersed while still containing almost 20% dry matter. The relation between Flours 6 and 11 is in agreement with the results obtained by Gortner and Doherty (9).

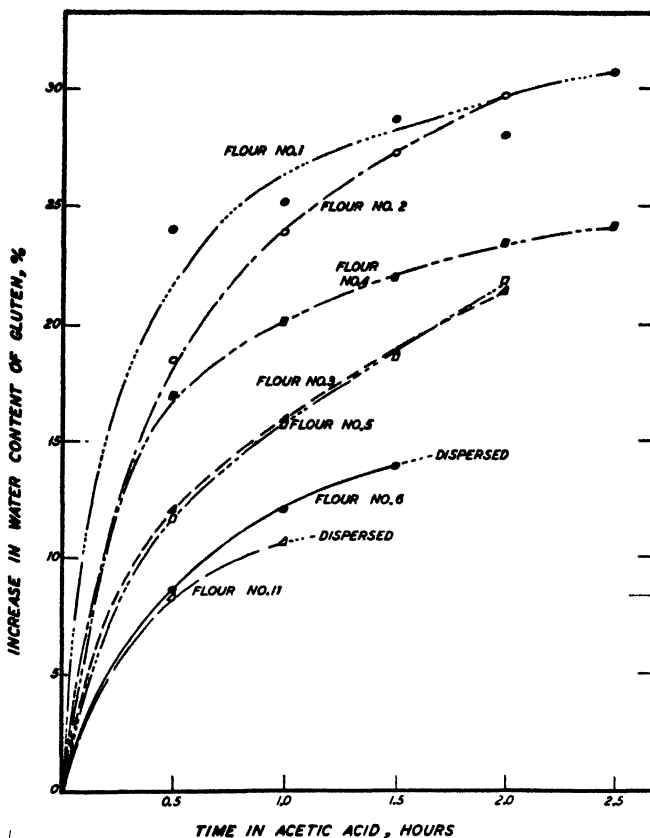


FIG. 2. The hydration capacity of gluten in 0.1 N acetic acid.

The enormous hydration capacity of the poor quality gluten has not, as far as we know, been reported before. It has been noted that the gluten from deteriorated flour disperses less readily in sodium salicylate than does that from high quality flour (19). The present results show that this is true in acid solutions also, and that a poor quality, freshly milled flour yields gluten similar to that of deteriorated flour, although the hydration capacity seems to be even greater at this low pH (approximately 3.0). The results obtained with the gluten from flour No. 3 are of particular interest, since this sample was just as poor in quality and hydration at higher pH values as No. 1 or No. 2, but did not exhibit the very excessive swelling of these two in acetic acid. This suggests not only that the original source and variety of the material (No. 3 was from originally high quality wheat) may affect

the swelling, but also that the factors determining the rate and extent of swelling in acid are different from those determining the hydration at higher pH values.

Effects of Acid and Salt Concentration on Gluten Swelling

That differences in the concentration of acids and salts present in gluten do not determine its physical properties has been established (9). Fisher and Halton (8), however, found that if 2% sodium chloride solution were used to wash out the gluten, differences in swelling by the Berliner method (2) disappeared. In order that our results should be comparable to those obtained by others, it was necessary to show that the differences obtained with glutes using 0.1 *N* acid would be obtained with more dilute acid and with varying concentrations of salt. Experiments were therefore carried out with Flours 1 and 6, which represented the two extremes of gluten quality. The results are presented in Table II. The glutes were washed with salt solutions of the concentration present in the acid used for swelling tests.

TABLE II

EFFECT OF ACID AND SALT CONCENTRATION ON HYDRATION OF GLUTEN, 1 HR. IMMERSION

Effect of acid			Effect of salt		
Acid concentration, normality	Increase in water, %		Salt concentration, %	Increase in water, %	
	No. 1	No. 6		No. 1	No. 6
0.001	3.0	3.8	0	25.1	12.5
0.01	12.1	10.1	0.05	22.3	—
0.02	16.5	10.9	0.1	15.2	5.0
0.05	19.5	12.3	0.2	11.9	2.0
0.1	21.8	12.5	0.5	3.0	0.7
0.5	24.8	12.3	1.0	1.5	0.4

Note: Ash in flour, %: No. 1, 0.67; No. 6, 0.40.

Ash in dry gluten, %: No. 1, 0.84; No. 6, 0.58.

The greatest differentiation between the two glutes was obtained at the highest acid concentration but the maximum swelling of the better gluten was in 0.1 *N* acid. At all concentrations except 0.001 *N* the greater hydration capacity of No. 1 was evident. Very low concentrations of sodium chloride reduced the swelling of the glutes, but their relative positions were maintained. The differences in the original properties of the gluten could not be due to the salts present, since the gluten that swelled the more was decidedly higher in ash.

It is concluded, therefore, that the differences noted among the glutes (Fig. 2) would have been obtained had the methods of Gortner and Doherty (9) been followed exactly.

Berliner and Koopman Method

The method of Berliner and Koopman (2) involves the same principles as does that of Gortner and Doherty (9), except for the method of measurement. Berliner and Koopman consider that a high swelling number indicates a good quality gluten. Gluten balls from Flours 1 and 6 were tested using this method, as outlined by Fisher and Halton (8). One-gram pieces of wet gluten were used and swelling was permitted to proceed for 5 hr. The results are presented in Table III.

TABLE III
SWELLING NUMBERS OF GLUTEN (BERLINER AND KOOPMAN)

Time, hr.	No. 1	No. 6	Time, hr.	No. 1	No. 6
0			2.0	27.1	7.8
0.5	11.8	4.1	2.5	30.0*	9.8
1.0	17.6	5.1	3.0	—	11.1
1.5	23.7	7.7	5.0	—	16.9

* Maximum possible to measure.

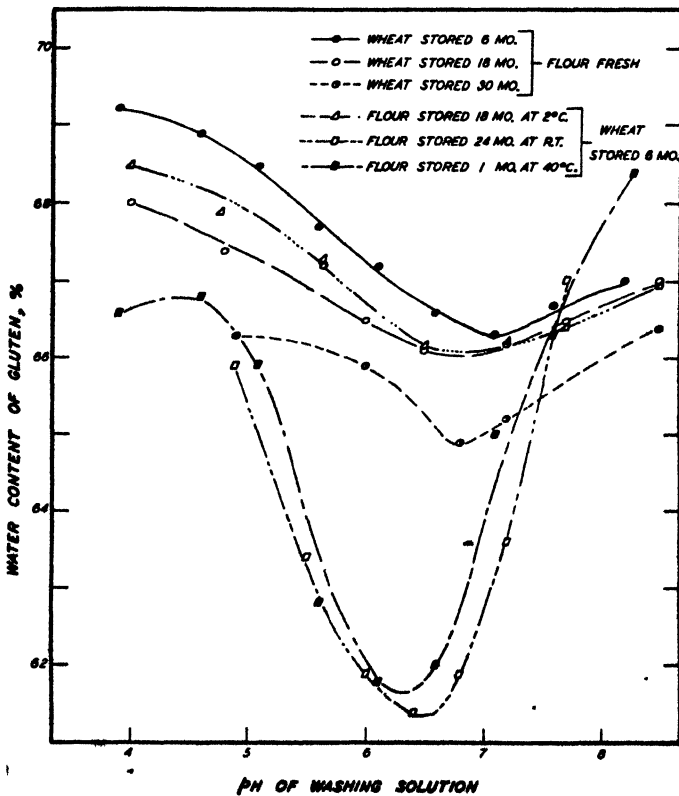


FIG. 3. The effect of pH on the hydration of gluten from stored flour and wheat.

By the end of 2 hr. the gluten from both flours had disintegrated to a considerable extent but measurements were continued. It is obvious that the swelling number does not represent the real volume of the swollen gluten as a large amount of water is entrapped among the pieces of gluten. The difference in behaviour of the two glutes agrees with the results obtained with the other method, and according to Berliner and Koopman shows that the gluten from flour No. 1 is decidedly the better of the two. This is, of course, incorrect. At the end of 22 hr. the gluten from flour No. 6 was dispersed, but that from flour No. 1 was granular. The particles were fine and did not settle readily. This behaviour is of the same type as is obtained when dispersion of similar glutes in sodium salicylate is attempted.

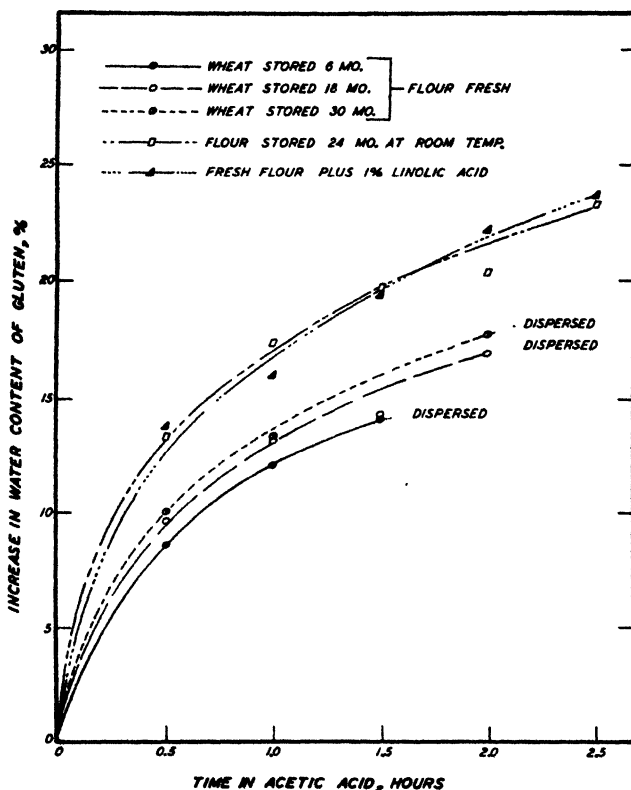


FIG. 4. The hydration capacity of gluten in 0.1 N acetic acid as affected by storage of wheat and flour.

*Effect of Aging of an Individual Flour on Water Absorption of Gluten**

The results in Figs. 1 and 2 showed a marked difference in the physical properties of gluten from fresh and deteriorated flours. Each of these flours was of different origin, however, and equally large differences were obtained with relatively fresh flours (compare Nos. 2 and 6). Flour No. 6 was therefore studied over a period of 24 months, to determine the changes that take place

in aging. A portion of this flour was artificially aged (see Material) and used in a similar study.

The results of the gluten washing tests are given in Fig. 3 and of the gluten swelling tests in Fig. 4. There was insufficient of the artificially aged flour for the latter test.

Two years' storage of the flour resulted in pronounced changes in the physical characteristics of the gluten; these changes resulted in a gluten very similar to those from any of the aged flours illustrated in Figs. 1 and 2. Artificial aging for one month at 20% moisture and 40° C. had the same effect. As with the poor quality glutes of Flours 1, 2, and 3, both the hydration capacity in buffer solutions and the pH at minimum absorption were reduced with aging. The glutes were coarse and open, lacked extensibility, and were very short. The loaf volume of the bread baked from this flour (naturally aged) had decreased by nearly 30% from that of the original.

The results also show that storage in the form of wheat resulted in gradual changes in the properties of the gluten but that these changes were much less than when Flour was stored. A small amount of the flour from this

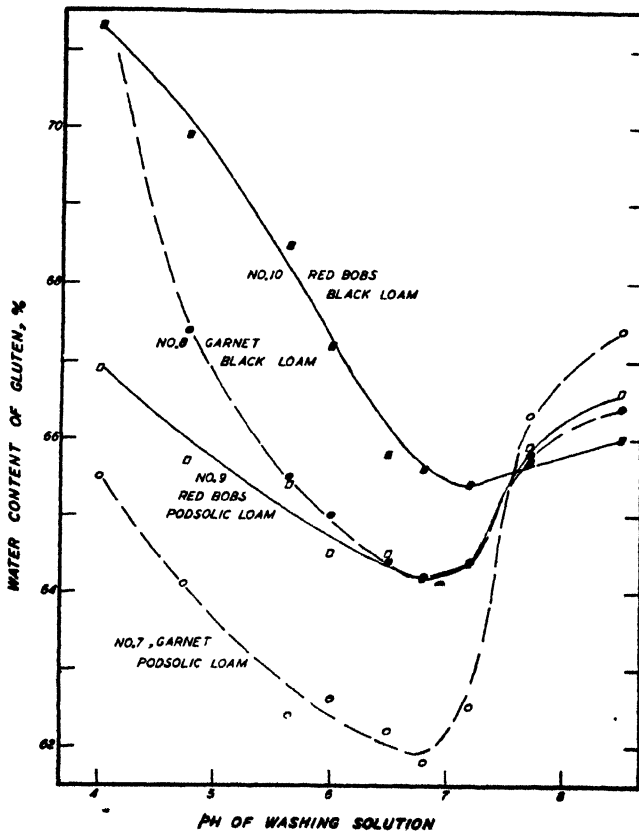


FIG. 5. The effect of origin and variety of wheat on the hydration of gluten.

wheat had been stored for 18 months in a refrigerator at 2° C. and was used in gluten washing studies. The results (Fig. 3) show that the change in gluten properties was small as compared with storage of the flour at room temperature.

Effect of Variety and Origin of Wheat on Gluten

The results in Figs. 1 and 2 showed also that different samples of freshly milled flour yielded glutens of widely different qualities. Much of the work done in this laboratory recently has been concerned with differences in the quality of wheat produced on two types of soil common in Alberta—the fertile black loam typical of the Edmonton district, and the relatively infertile podsollic loam, typical of much of the northern and western parts of the province. In general the quality of wheat from the former is good, and of that from the latter, poor. This is, to a considerable extent, due to differences in protein quantity, but it has been evident that there are also differences in protein quality. These differences are illustrated in Fig. 5. Flours 7 and 9 are from Garnet and Red Bobs wheat, respectively, grown on the podsol, whereas Flours 8 and 10 are from the same varieties grown on the black loam

Not only do these results illustrate the differences in the gluten from wheat grown on the two soils, but also the differences in the glutens from the two varieties. These differences are quite in accord with baking results, except that the protein quantity factor has here been removed.

Effects of Ether Extraction and Linolic Acid on Gluten

The results presented in Fig. 4 suggested that the changes in physical properties of gluten with flour deterioration are paralleled by the changes resulting from the addition of linolic acid. The effect of fatty acids on gluten quality has been discussed in an earlier paper (19). It was concluded that the accumulation of these acids was only one factor in determining the quality of aged flour, and that the breakdown of a complex between the protein and the more insoluble lipoids was more important, at least as far as baking quality was concerned.

Samples of Flours 3 and 6 were ether extracted (19) and the gluten then washed, using solutions of various pH values. Linolic acid was added to other samples of ether extracted flour in such quantity as to compensate for the fatty acids removed by ether extraction. The results of these tests are given in Fig. 6.

Ether extraction increased the hydration of gluten from flour No. 6 at all pH values, and of the gluten from flour No. 3 at all values below pH 6.5. This improvement in hydration is attributed to the removal of fatty acids by the extraction. The gluten from extracted flour No. 3 was still coarse, open, and short, indicating that the extraction had not removed all the effects of aging. Such extraction, therefore, did not bring the gluten of flour No. 3 back to its original properties, as this flour was originally of excellent quality, quite comparable to flour No. 6. The addition of linolic acid to compensate

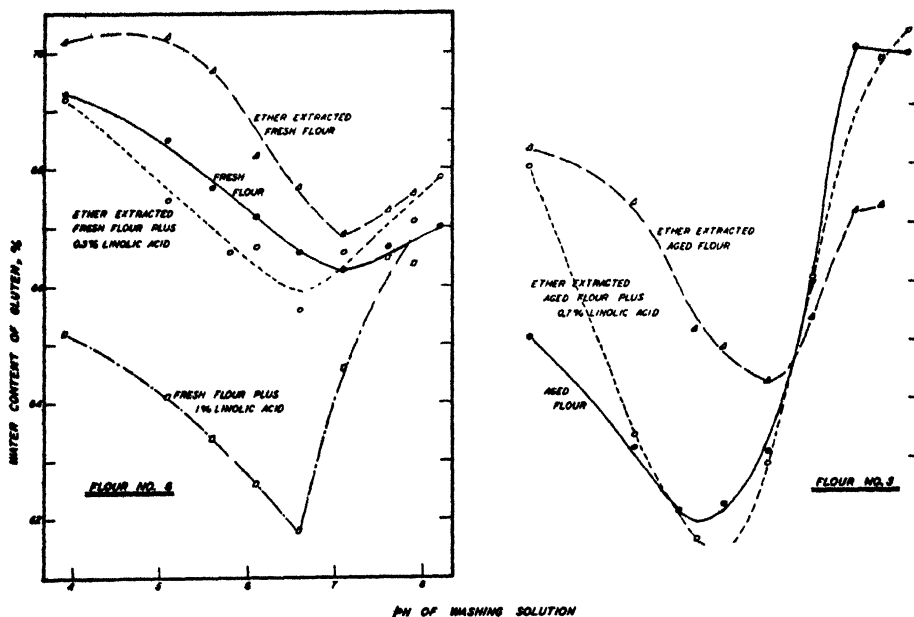


FIG. 6. The effects of ether extraction of flour and addition of linolic acid on the hydration of gluten from fresh and aged flour.

for the extracted acids made the gluten from extracted flour comparable to that of the gluten from the unextracted.

These results substantiate the earlier hypothesis (19), that there are two factors determining the quality of the gluten from deteriorated flour, and that the more insoluble lipoidal substances present in the flour compete with fatty acids in determining the type of gluten complex formed on washing. If the fatty acids are extracted, gluten quality is improved, but unless the important lipoids are available, good quality gluten cannot be obtained.

Discussion

The conception of gluten as a complex of protein and lipoids has been discussed in an earlier paper (19) and by others (3). It was suggested that the physical changes in gluten that occur as flour ages are due to two factors: first, the breakdown of the complex and second, the accumulation of end products, particularly the unsaturated fatty acids. As a result of further studies this hypothesis needs some modification. "Gluten is a protein-lipoid complex but this complex is apparently formed when a dough is made or the gluten is washed from the flour. It is, therefore, probably incorrect to speak of the breakdown of this complex with aging of the flour, since it is more likely that a change in the lipoidal substances themselves renders them incapable of conferring upon the gluten mass the properties of coherence and extensibility. As flour ages the breakdown of lipoidal compounds also results in the accumulation of fatty acids, which compete for the adsorption bonds in the formation of the gluten."

If a particular type of lipoidal substance is necessary to produce high quality gluten, the results obtained in this study may be interpreted in the following way. High quality flour produces coherent, extensible, and elastic gluten because the essential lipoids are adsorbed on the protein of the gluten. When fatty acids are added to a high quality flour, they compete with the lipoids for the adsorption bonds and, if present in sufficient quantity, replace the other lipoids to such an extent that the gluten loses much of its coherence and extensibility. As a flour ages, the essential lipoids break down and fatty acids accumulate. Eventually the flour yields gluten which is coarse, open, and short. When the fatty acids are extracted, the quality of the gluten is improved because the acids no longer compete with the remaining lipoids, but the original quality is not regained because the breakdown of the original lipoids has proceeded too far.

In this study, another type of flour was encountered. From this (flour No. 2) good quality gluten was not obtainable even at the time of milling. It seems probable that the lipoidal substances necessary to produce a high quality gluten complex were not available in this flour; that is, they had not been metabolized.

These conclusions permit the acceptance of the hypothesis suggested by Bungenberg de Jong (5). If gluten is considered as a single colloidal complex made up of various protein components plus one or more lipoidal substances, this general hypothesis fits all the results obtained in the present study. A complex made up only of protein would have an isoelectric point at the point of balance between positive and negative charges on the components. The presence of lipoidal substances alters this point, its exact position being determined by the amounts and proportions of the various lipoids. Our results indicate that in a high quality gluten this point, which probably should not be called an isoelectric point except that the term serves to indicate a point of minimum swelling, is at or near pH 7.0. Any alteration in the balance of the lipoids taking place as the flour ages results in this point shifting to a lower pH. From Bungenberg de Jong's results (5), it seems probable that the protein complex alone would have an isoelectric point at approximately pH 6.0, and this point is about the lowest obtained in the present study. This agreement may be fortuitous, however, since fatty acids at least, and probably some unaltered essential lipoids, were present in all of the flours used in the present study.

The results of the gluten swelling experiments carried out on these flours are determined, it is believed, by different factors, since pH 3.0 is well on the acid side of the isoelectric point of any of the components. The results of this test give an indication of the dispersibility of the gluten rather than any other quality factor, the most easily dispersed gluten exhibiting the slowest rate of swelling and the lowest hydration at which coherence is maintained:

Only a few remarks have been made regarding the gluten from soft wheat flour. There is no doubt that this is different from hard wheat gluten but too little has been done to characterize it accurately. The comparison of

gluten from different types of wheat is to be the object of another study. The quality of individual flours used in this study varied enormously. The study of gluten reveals great differences, but whether or not such gluten studies can be used in differentiating flours of similar type and general quality remains to be determined.

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EFFECTS OF TALC DUSTS CONTAINING PHYTOHORMONE, NUTRIENT SALTS, AND AN ORGANIC MERCURIAL DISINFECTANT ON THE ROOTING OF HERBACEOUS CUTTINGS¹

BY N. H. GRACE²

Abstract

Cuttings of *Coleus Blumei*, varieties of *Chrysanthemum* and species and varieties of *Iresine* were treated with a series of talc dusts containing naphthylbutyric acid, nutrient salts, and ethyl mercuric bromide and then were propagated in sand in the greenhouse. Naphthylbutyric acid treatment increased the number of roots per rooted cutting, and its combination with the mixture of nutrient salts increased fresh root weight of *Coleus* cuttings. Organic mercury treatment increased, by about 5%, the number of *Chrysanthemum* cuttings that rooted, and increased the number of roots on *Iresine* cuttings. Beneficial effects from talc treatment alone were a feature of the results. Differential reactions to both talc and organic mercury treatments were shown by closely related varieties.

Earlier communications have reported on the effects of treatment of plant stem cuttings with talc dusts containing phytohormone, nutrient salts, and disinfectant chemicals (2, 3, 6, 7, 9, 10). This communication describes the results of such treatment on cuttings of three genera of herbaceous plants.

Experimental

The factorial series of talc dusts used in these experiments has been described in detail in a recent article dealing with the responses of *Taxus* cuttings (9). The dusts contained a mixture of nutrient salts at concentrations of 0, 0.1, 1, and 10%, each taken separately in talc, and in combination with 0 and 50 p.p.m. ethyl mercuric bromide and 0, 250, and 1000 p.p.m. of naphthylbutyric acid. Cuttings* of three horticultural varieties (Margaret Waite, Lillian Godfrey, and Bronze Godfrey) of *Chrysanthemum indicum* L. were treated with the entire series of 32 dusts. *Iresine* (*I. Lindenii* Vanhoutte, *I. Herbstii* Hook. f., and *I. Herbstii* var. *aureo reticulata*) received all treatments except 250 p.p.m. naphthylbutyric acid. Cuttings of *Coleus Blumei* Benth. were treated with only 16 of the dusts. Treatments with 250 and 500 p.p.m. were omitted.

Cuttings were sprinkled with water and groups of seven of *Chrysanthemum* and *Iresine* and five of *Coleus* were dipped in dust to a depth of about one-half inch. Excess dust was shaken off and the cuttings were planted immediately in a relatively coarse brown sand (8); the frames were covered with factory cotton screens for the first week after planting. The experiments were made

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*Prepared cuttings of *Coleus* and *Iresine* were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood. Cuttings of *Chrysanthemum* were purchased from a local florist.

during the months January to March, 1940. During this period the temperature in the greenhouse ranged around 65° F.

All experiments were arranged according to the principles of experimental design, with treatments replicated and groups of cuttings arranged in random order in the medium. The random arrangement permitted comparison of three varieties of *Chrysanthemum* and two species and a variety of *Iresine* and the interactions between species and varieties and the various chemical treatments. Experiments included groups of untreated cuttings to permit consideration of the effects of talc treatment. The experiments required 1512 *Chrysanthemum* cuttings, 255 cuttings of *Coleus*, and 1638 of *Iresine*. All results were subjected to the analysis of variance procedure except where the data were too meagre to warrant such treatment. Data for counts of numbers of cuttings were subjected to the inverse sine transformation prior to statistical treatment (1).

Cuttings were removed approximately four weeks after planting and record was made of the number rooted, dead, and the number of roots. The length of root mass and fresh weight of plants and of roots were determined for *Coleus* and *Iresine*. Also, total root length of *Chrysanthemum* cuttings was determined and the mean root length calculated. The number of roots and the fresh plant and root weights were calculated on a "per rooted cutting" basis.

Results

The results of the analyses of variance of data are not given. The statistical treatment was closely similar to that described in detail for a previous experiment involving use of the same chemical treatments (9).

TABLE I
AVERAGE EFFECTS OF ETHYL MERCURIC BROMIDE ON THE PERCENTAGE ROOTING OF
Chrysanthemum CUTTINGS

Concentration of ethyl mercuric bromide, p.p.m.	<i>Chrysanthemum</i> varieties			Mean of treatments
	Bronze Godfrey	Lillian Godfrey	Margaret Waite	
0	70.5	41.1	11.6	41.1
50	75.0	44.7	17.4	45.7

Chrysanthemum

Effects of ethyl mercuric bromide on rooting are described in Table I in which the data are averages for all phytohormone and nutrient salt treatments. Although the average beneficial effect was only 4.6%, it was apparent in all three varieties. Marked differences in extent of rooting of these horticultural varieties also was evident.

Data comparing the responses of untreated and talc treated cuttings are given in Table II. Talc appeared to increase the rooting of two, and decrease rooting of one, of the varieties. Beneficial effects also were suggested by counts of numbers and lengths of root per rooted cutting.

TABLE II
EFFECT OF TALC TREATMENT ON RESPONSES OF *Chrysanthemum* CUTTINGS

Variety	Cuttings rooted, %		Number of roots per rooted cutting		Length of roots per rooted cutting		Mean root length, mm.	
	Untreated	Talc treated	Untreated	Talc treated	Untreated	Talc treated	Untreated	Talc treated
Bronze Godfrey	67.9	71.4	7.2	9.2	106	138	14.7	15.1
Lillian Godfrey	57.2	32.1	6.8	8.8	105	138	15.5	15.7
Margaret Waite	3.6	10.7						

Results of naphthylbutyric acid treatments on the number of roots per rooted cutting are given in Table III, in which the data are averages for all nutrient salt concentrations and treatments with and without organic mercury. The number of roots increased progressively with increase in concentration of naphthylbutyric acid; the increase was significant at the 1000 p.p.m. concentration. However, this significant increase in number of roots occurred in the absence of organic mercury, or when the 1000 p.p.m. concentration was in combination with both organic mercury and nutrient salts, particularly the 10% concentration of the latter.

TABLE III
AVERAGE EFFECTS OF NAPHTHYLBUTYRIC ACID ON THE NUMBER OF ROOTS PER ROOTED *Chrysanthemum* CUTTING

Naphthylbutyric acid in talc, p.p.m.				Necessary difference, 5% level
0	250	500	1000	
8.0	8.6	9.4	10.1	1.56

Coleus

Rooting of the talc treated cuttings attained 98%, that of the untreated, 93%. Naphthylbutyric acid treatment increased the number of roots per rooted cutting by 2.7 on the average, i.e., from 10.9 to 13.8. Interaction effects of phytohormone and nutrient salt treatments on fresh root weight per rooted cutting are described in Table IV in which the data are averages for treatments with and without organic mercury. The substantial increase

after phytohormone treatment is wholly attributable to the interaction between naphthylbutyric acid and nutrient treatments, particularly the 10% concentration.

TABLE IV

AVERAGE EFFECTS OF NAPHTHYLBUTYRIC ACID AND NUTRIENT SALTS ON THE FRESH ROOT WEIGHT PER ROOTED *Coleus* CUTTING, CG.

Naphthylbutyric acid in talc, p.p.m.	Nutrient salts in talc, %				Mean of naphthylbutyric treatments
	0	0.1	1	10	
0	10.9	9.8	8.6	9.4	9.7
1000	10.9	10.5	11.9	16.4	12.4

Necessary difference 5% level, for interaction: 3.8.

Iresine

The data in Table V indicate that talc treatment effected marked increase in rooting, number of roots, and fresh plant weight, and reduced mortality.

TABLE V

AVERAGE EFFECTS OF TALC TREATMENT ON RESPONSES OF *Iresine* CUTTINGS

Responses	Untreated cuttings	Talc treated cuttings
Number of cuttings rooted, %	40.5	95.6
Number of cuttings dead, %	58.0	4.1
Number of roots per rooted cutting	12.2	17.0
Fresh plant weight per rooted cutting, dg.	6.2	8.0

TABLE VI

INTERACTION EFFECTS OF TALC TREATMENT AND SPECIES AND VARIETIES OF *Iresine* ON THE FRESH PLANT WEIGHT PER ROOTED CUTTING, DG.

—	<i>I. Lindeni</i>	<i>I. Herbstii</i>	<i>I. Herbstii</i> var. <i>aureo reticulata</i>
Untreated cuttings	5.9	7.1	5.8
Talc treated cuttings	6.7	8.6	8.9

Necessary difference, 5% level: 1.3.

Differential effects of talc treatment on fresh plant weight of species and varieties are given in Table VI, significant increase in plant weight from talc treatment being shown by *I. Herbstii* and its variety *aureo reticulata* but not by *I. Lindeni*.

Naphthylbutyric acid treatment, on the average, increased the number of roots per rooted cutting from 15.2 for controls to 17.4 and 18.4 after treatment with the 500 and 1000 p.p.m. concentrations respectively, the necessary difference being 0.89 for the 5% level of significance.

TABLE VII
AVERAGE EFFECTS OF ORGANIC MERCURY ON THE NUMBER OF ROOTS PER
ROOTED *Iresine* CUTTING

Concentration of ethyl mercuric bromide in talc, p.p.m.	<i>I. Lindeni</i>	<i>I. Herbstii</i>	<i>I. Herbstii</i> var. <i>aureo reticulata</i>	Mean of organic mercury treatments
0	14.2	16.9	17.4	16.2
50	16.7	19.2	17.4	17.8

Necessary difference, 5% level: 1.78.

The effects of organic mercury treatment are given in Table VII in which data for the number of roots per rooted cutting are averages over all phytohormone and nutrient salt concentrations. A highly significant increase of 1.6 in the number of roots resulted from organic mercury treatment in *I. Lindeni* and *I. Herbstii*. Treatment failed to affect the number of roots per rooted cutting of *I. Herbstii* var. *aureo reticulata*. Data for the fresh root weight per rooted cutting indicated that the variety had a substantially greater root weight than the *I. Lindeni* and *I. Herbstii* which did not differ in this respect.

Discussion

Naphthylbutyric acid treatment effected a general increase in the number of roots per rooted cutting. Combination of this growth stimulating chemical with a mixture of nutrient salts substantially increased fresh root weight of *Coleus* cuttings. Combination of the 1000 p.p.m. concentration of this chemical and 10% of nutrient salts in talc has already been shown to affect the length of new growth of *Taxus* cuttings (9). Effects of nutrient salt treatments of these herbaceous cuttings were neither as numerous nor as pronounced as with cuttings of some other species (2, 6, 9, 10).

Organic mercury treatment increased rooting of *Chrysanthemum* cuttings, though the extent of the effects was small. Organic mercurial treatments have been shown to increase the rooting of other plants (2, 10). Effects of 50 p.p.m. ethyl mercuric bromide on number of roots per rooted cutting in *Iresine* are similar to those of an earlier experiment in which the same concentration of the phosphate was used (2). It may be concluded that organic mercury treatment favourably affects responses of cuttings of only certain plants, and that there may be differences between varieties and closely related species. Although the extent of the effects is usually small, it may be

recalled that rooting of *Physocarpus* cuttings was increased as much as 19% by organic mercurial treatment (2).

The beneficial effects of talc treatment stand out as a feature of the results. Similar effects on responses of cuttings of other plant species have been reported (4, 5, 7, 11, 12). Although each of the three genera considered demonstrated increased rooting from talc treatment, one variety of *Chrysanthemum* and one of *Iresine* failed to respond favourably. Differential effects from talc treatment have been noted with spruce cuttings (7). Beneficial effects from talc treatment have been attributed, in part, to water relationships (4, 11). In these experiments relative humidity tended to be low in the greenhouse and there were some drafts of warm air owing to unit heaters. Such conditions might be particularly favourable to talc treatment.

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STRAWBERRY ROOT ROT IN RELATION TO MICROBIOLOGICAL CHANGES INDUCED IN ROOT ROT SOIL BY THE INCORPORATION OF CERTAIN COVER CROPS¹

BY A. A. HILDEBRAND² AND P. M. WEST³

Abstract

Strawberry plants, variety Premier, were grown in naturally-infected root rot soil in which consecutive "crops" of several agricultural plants had been turned under, and in other lots of the same soil that had been steam sterilized or fertilized with barnyard manure. On examination of their roots it was found that the incidence and severity of root rot were closely correlated with soil treatment. Plants grown in sterilized soil remained free from disease as did those of the soybean series until the third season when they were slightly affected. Plants in the manure, corn, red clover, timothy, and untreated soil series all became diseased, the severity of attack increasing in the respective series in the order named.

Although roots of the various cover crops were found to contain representatives of several different genera of fungi, a specific fungus was dominant in each as was the nematode, *Pratylenchus pratensis*, in timothy and clover. This build-up of specific organisms appeared to be correlated with the incidence and severity of the disease in the roots of the strawberry plants that followed in the respective series.

However, in strawberry plants grown in the variously treated soils, fungal infection was negligible and, on the whole, not related to that of the preceding cover crop. An exception to this was the heavy infection by the mycorrhizal fungus (*Rhizopagus* sp.), following timothy and corn, but a lack of correlation, in many cases, between the presence of the fungus and discoloured and necrotic tissue indicated that other agencies might be responsible for the injury. In timothy and red clover treated soils, nematodes, particularly *Pratylenchus pratensis*, might have been an important factor.

Fewer bacteria were found adjacent to roots of healthy plants than to those of diseased ones. Qualitative differentiation on the basis of nutritional requirements indicated a striking relationship between the incidence of certain groups of bacterial isolates and the severity of disease attack. The equilibrium between presumably "harmful" bacteria and the innocuous, normally occurring rhizosphere types is designated the Bacterial Balance Index. There are marked differences in the microbiological equilibria of the different soil series; increased severity of root rot is associated with a fall in the Bacterial Balance Index.

Introduction

Since the control of strawberry root rot obviously cannot be carried out under practical conditions by soil sterilization, and since satisfactory resistant varieties are not available, the most promising solution of the problem would appear to be a biological one. In a recent comprehensive review of the literature relating to soil-borne fungi and the control of root diseases, Garrett

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(2) discusses very fully "the growing promise of biological control as a practical agricultural method" and cites a number of instances in which the method has been used with success in the control of certain soil-borne pathogens (8, 1, 10, 5). Because of such successes, the possibility of biological control as applied to strawberry root rot has been explored, efforts having been directed towards inactivation of suspected pathogens in the soil before the growing season. In this paper are presented results of experiments, carried out under greenhouse conditions, that show that incidence and severity of the disease can be modified by certain green-manurial treatments and that these modifications are correlated with changes in the microbiological equilibria of naturally infected root rot soil.

Description of Experiment

The soil used in the experiment, a light sandy loam, was obtained in the early fall of 1936 from a strawberry plantation which had failed that year because of high mortality of plants following attack by root rot. Soil from this heavily infected area after being thoroughly mixed was transferred to the greenhouse in 9-in. clay pots.

During the winters of 1936-37, 1937-38, and 1938-39, successive crops of tomatoes, corn, red clover, soybeans, oats, rye, timothy, and rape, respectively, as well as combinations of these plants in rotational schemes, were grown in different lots of the root rot soil. At the peak of their stage of succulence, the plants were chopped up finely and incorporated with the soil in which they had grown. In a winter season three or four crops of the faster growing plants like soybeans or tomatoes could be turned under but only one or two sowings of a slower growing plant like timothy in the same period of time. In a series like red clover-oats-corn, it was possible to complete the rotation only once during a winter. Additional series in the experiment included those in which well rotted manure and weeds instead of agricultural plants were added to the root rot soil. Two other series included untreated (control) root rot soil and the same soil sterilized with steam before each summer planting of strawberries. Altogether at the outset, the experiment included 16 different series with three pots to a series. At the end of the second summer, however, results indicated that nothing was to be gained by continuing nine of the series and they were discontinued. In this paper data are presented for the seven remaining series that showed most interesting and promising results, namely, timothy, soybeans, corn, manure, red clover, sterilized root rot soil, and untreated root rot soil.

In the summers of 1937, 1938, and 1939 the pots of soil were transferred from the greenhouse in the third week of August to outdoor plots of strawberries, variety Premier, where they were so placed that four vigorous runner plants could be struck in each pot. Each summer the pots were left outdoors until about October 1; at that time they were transferred to the greenhouse. There the strawberry plants were allowed to continue their growth until the third week in November, by which time they were approximately three

months old. They were then removed from the soil for examination and dry weight determination.

At certain stages of the experiment selected material from the roots of the strawberry plants grown in the soils subjected to the different treatments, and from those of the cover crop plants, were examined microscopically. In addition, chemical tests of the soil were made using the Morgan universal rapid soil testing system, pH determinations being made at the same time.*

In November, 1939, when the roots of the strawberry plants grown in the variously treated soils were showing the most marked differences, it became possible to broaden the scope of the investigations to include bacteriological studies. Bacterial isolates were obtained from the rhizospheres of the strawberry plants of certain series, following procedures previously outlined by Lochhead (7) and by Taylor and Lochhead (11). Qualitative analysis of the bacterial flora and determinations of bacteriological equilibria were made according to the methods of West and Lochhead (15).

Macroscopic Examination of Roots

STRAWBERRY PLANTS

In November of each year after the strawberry plants had completed the three-month period of growth for the season, they were removed from the variously treated soils and after being thoroughly washed were spread out in water against a white background for macroscopic observation. Following this, material was selected for microscopic examination (second year) and finally dry weight determinations were made. Since each series comprised three pots with four strawberry plants in each, it was possible to make observations and comparisons recorded below on a basis of 12 plants per series per season. Dry weights of the plants for each year are presented in Table I;

TABLE I

DIFFERENCES IN WEIGHTS OF ROOTS OF STRAWBERRY PLANTS GROWN IN TREATED AND UNTREATED ROOT ROT SOIL

Series No.	Treatment of root rot soil	Dry weight of 12 roots, gm.			Aggregate weights
		1937	1938	1939	
14	Steam sterilized	(1)† 72.0	(2) 33.9	(3) 25.6	131.5
4	Soybeans	(4) 35.0	(8) 25.0	(12) 20.1	80.1
16	Barnyard manure	(1) 31.3	(2) 23.4	(3) 16.2	70.9
1	Corn	(4) 36.0	(7) 19.0	(10) 12.6	67.6
3	Red clover	(3) 37.0	(6) 15.6	(9) 4.8	57.4
7	Timothy	(2) 20.0	(4) 10.2	(6) 8.7	38.9
15	Untreated (control)	23.0	7.8	3.4	34.2

† Figures in parentheses indicate number of times cover crops were turned under and number of repetitions of other treatments.

* Within the range of accuracy obtainable by this method no significant chemical changes were noted, except that the pH of the root rot soil, originally 7.0 to 6.8, was lowered to and remained at 5.8 for some time following the turning under of each crop of soybeans.

some appreciation of the qualitative differences between roots of the various series, which are described below, may be gained from Plate I, Figs. 1 to 7.

Series 14. Steam Sterilized Soil

Plants grown in the root rot soil that was sterilized each year just before the runners were struck, consistently developed virtually flawless roots. The white, silky lustre of even the finer laterals, together with their highly turgid condition gave evidence not only that these roots were free from disease but also that, physiologically, they were still functioning normally. As reference to Table I will show, the aggregate weight of the roots of this series over the three-season period was 131.5 gm. This weight exceeds by 51.4 gm. that of the roots of the soybean series which ranked second best. It will be noted further that the weight of the plants in this series decreased in successive seasons. This decrease in root production cannot be attributed to an increasingly important disease factor because from the pathological standpoint the roots grown in 1939, which weighed only 25.6 gm., were just as healthy as those grown in 1937, which weighed 72 gm.

Series 4. Soybeans

From the standpoint of both bulk and health, the roots of strawberry plants of the soybean series approached most closely those grown in sterilized soil. In 1939, they showed some discoloration but were still markedly superior to roots of any of the remaining series.

Series 16. Barnyard Manure

It will be noted in Table I that the aggregate dry weight of roots of the manure series was only 9.2 gm. less than that of roots of the soybean series. Despite this rather close parallelism in bulk of root production the plants of the manure series from the standpoint of health did not compare at all favourably with those of the soybean series. Whereas the latter showed little discoloration even in the third season, the former each year showed an almost general reddish-brown discoloration as well as a considerable number of definitely diseased main roots and laterals. The impression was gained from plants of this series, that during or following a period of apparently rapid and extensive root formation, some agency in the soil had brought about relatively quickly the condition of the roots described above. That such condition in this or other series is to be distinguished from that accompanying or resulting from normal senescence would seem to be proved by the fact roots of plants of the same age grown in sterilized soil showed no signs of a

Series 1. Corn

In 1937, the roots of the strawberry plants grown in the soil after the incorporation of four crops of corn, were fairly good. In bulk they even slightly exceeded those of plants following the soybean series. However, in 1938 and 1939 their condition was about midway between that of plants of the best and poorest series. They showed general brownish discoloration and a relatively high proportion of dead and dying roots.

PLATE I



¹ Roots of three month old Premier strawberry plants, 1939 series showing correlation between incidence and severity of root rot and manurial and other treatments of naturally infected soil FIG 1 Root rot soil untreated FIGS 2, 3, 4, AND 6 Root rot soil plus 9 red clover, 6 timothy, 10 corn and 12 soybean cover crops, respectively FIG 5 Root rot soil plus 3 applications of barnyard manure FIG 7 Root rot soil sterilized with steam

Series 3. Red Clover

The results observed in this series proved to be very interesting and most surprising, especially when they are compared with those recorded for the soybean series. Since both are leguminous plants, it might reasonably be expected that their effects would be the same. The results obtained in 1937 suggested that such would be the case because that year the weight of the roots of the strawberry plants grown after the turning under of the first three crops of red clover slightly exceeded that of the plants of the soybean series. Pathologically there was little at the time to differentiate the roots of the two series. In 1938, however, the roots of the red clover series had dropped from second best in the scale to third poorest. Their discoloured and necrotic condition suggested an advanced stage of root rot. In 1939, the 12 strawberry runner plants struck in the soil with which nine crops of red clover had then been incorporated, developed so few roots that their total dry weight was only 4.8 gm. The few roots that had developed were without exception either badly discoloured or in an almost completely necrotic condition. In fact, it was difficult to find sufficient suitable material for microscopic examination.

Series 7. Timothy

Every year, roots of strawberry plants following timothy were very poor. In 1937, after only two crops of timothy had been turned under, the strawberry plants developed roots that were even poorer than those of plants grown in the untreated root rot soil. In 1939, three of the strawberry runner plants, like the 12 in the red clover series of the same year, produced almost no roots at all. The remaining nine runners developed to a slightly greater extent the semblance of a root system which at the time of the examination had reached an advanced stage of disintegration. It will be noted that the aggregate weight of the roots produced by all 36 plants of the timothy series over the three-year period was only 38.9 gm. These plants gave the impression that some powerful agency in the soil was not only inhibiting root development but was contributing to the destruction of the relatively few roots that were formed.

Ser 15. Untreated (Control) Soil

In 1937, only the plants of the timothy series were poorer than those of the unsterilized soil or control series. By 1939, the soil had become so "sick" for strawberries that the survivors of the few roots that were formed weighed only 3.4 gm. The total weight of the roots produced by the 36 plants of this series for the three-year period was considerably less than half and only about one-quarter of that of the roots of the soybean and sterilized soil series, respectively.

COVER CROP PLANTS

In general, roots of the cover crop plants received only passing attention unless something unusual in their condition invited closer scrutiny. In corn no diminution of growth was noted in successive crops and the roots of

plants of the tenth sowing appeared to be as healthy as those of the first. In timothy and red clover, the sowings of the third winter season yielded less general bulk of plant than those of the first two years. The first eight sowings of soybeans resulted in excellent stands of plants, the roots of which never showed more than slight indications of discoloration and necrosis. However, the ninth sowing (i.e., the first of the third winter season) was almost a complete failure. A considerable number of the seeds germinated but gradually most of the young plants died, leaving finally only a few weak survivors. The roots of the latter were badly rotted and microscopic examination of necrotic tissue revealed extremely heavy infection by *Thielaviopsis basicola* (Berk.) Ferraris. It was thought at first that the fungus might have been introduced in or on the seed but, as attempts to culture it from a surplus of the latter proved unsuccessful, there seemed little doubt that the organism was already present in the soil. There is no reason for believing, however, that *T. basicola* was entirely responsible for the failure of this particular planting of soybeans. Rather, it would seem that some fault in the seed was a contributing factor. In three subsequent plantings, in which a new lot of seed was employed, excellent stands of plants were obtained, the roots of which though readily showing visible evidence of black root rot were, however, never affected severely enough to cause appreciable inhibition of growth of above-ground parts.

Microscopic Examination of Roots

In regard to the microscopic examination of the roots of the various plants in the experiment, one of two procedures could have been followed. Material for examination could have been selected either at periodic intervals throughout the experiment or at a comparatively late stage when it might reasonably be expected that an end point had been reached. Although the first mentioned procedure would have been highly desirable from the standpoint of detecting and following gradual microbiological changes, nevertheless, its adoption would have entailed the examination of a much greater amount of material than the second. As this would have required much more time than was available for this phase of the experiment, the second was adopted.

ROOTS OF COVER CROP PLANTS

Material for examination was selected from roots of the cover crop plants of the first sowing of the third winter season, i.e., the fifth, seventh, eighth, and ninth crops of timothy, red clover, corn, and soybeans, respectively. Employing the technique that had been used with success in similar investigations (12, 3, 6) sufficient material was prepared to permit the examination of a minimum of 1000 mm. of root tissue of each cover crop. An attempt was made to select material that was not only comparable in age but which appeared to be in a seemingly similar pathological condition. Attention was confined primarily to rootlets showing the earlier stages of discoloration and necrosis since in such the chance of primary parasites being obscured by

TABLE II
INFECTION VALUE OF FUNGI AND INCIDENCE OF NEMATODES PER 1000 ML. OF ROOT TISSUE OF COVER CROP PLANTS

Series No.	Cover crop	Fungi observed and infection values assigned						Total infection value of fungi	Nematodes	
		<i>Rhizoctonia Solani</i>	Phycomycetous mycorrhizal fungus	<i>Asterocystis</i>	Unidentified fungi	<i>Thidiaspiopsis bastarda</i>	<i>Pythium</i> spp.	<i>Helminthosporium</i>	Number	Kind
2	Tomatoes (5)*	794	79	31	61	—	—	—	965	48
4	Soybeans (9)	—	Trace	24	Trace	2695	14	—	2733	73
1	Corn (8)	—	307	43	583	—	1483	139	2555	70
7	Timothy (5)	—	415	73	125	—	154	16	783	732
3	Red clover (7)	—	1897	—	—	—	—	—	1897	960

* Figures in parentheses denote number of times cover crops have been turned under.

TABLE III
INFECTION VALUE OF FUNGI AND INCIDENCE OF NEMATODES PER 1000 ML. OF ROOT TISSUE OF STRAWBERRY PLANTS GROWN IN TREATED AND UNTREATED ROOT ROT SOIL

Series No.	Treatment of root rot soil	Fungi observed and infection value assigned						Total infection value of fungi	Nematodes		Dry weight of roots, gm.
		<i>Rhizoctonia</i> Orchid type	<i>Solani</i> type	<i>Asterocystis</i>	<i>Alternaria</i>	<i>Pythium</i>	Phycomycetous mycorrhizal fungus	Unidentified fungi	Number	Kind	
7	Timothy (4)*	3	Trace	—	—	—	2067	Trace	536	<i>Pratylenchus pratensis</i>	10.2
1	Corn (7)	12	—	—	—	—	1617	—	—	—	19.0
15	Untreated (control)	—	8	—	Trace	9	1341	Trace	19	Saprophytic spp.	7.8
16	Barnyard manure (2)	—	—	—	—	—	828	—	—	—	23.4
4	Soybeans (8)	—	—	24	—	Trace	216	—	2	Saprophytic sp.	25.0
14	Steam sterilization (2)	Trace	—	—	—	—	—	—	—	—	33.9

* Figures in parentheses indicate number of times cover crops turned under and repetitions of other treatments.

secondary saprophytic invaders would be less than in rootlets in more advanced stages of disintegration. Starting at one extremity of a stained specimen, successive microscopic fields were examined and micro-organisms (chiefly fungi) observed in each field were assigned an empirical value based on the extent and degree to which the tissues were invaded. Summation of values for all microscopic fields in the examination of 1000 mm. of root tissue gave the total infection value for the fungal organisms observed. In the case of nematodes it was not necessary to assign infection values since for purposes of comparison the actual number of these organisms present in the tissues of roots of the different series could be counted accurately. In Table II are shown the infection values assigned the fungi and the numbers and kinds of nematodes observed in the roots of the respective cover crop plants.

Analysis of the data summarized in Table II reveals some interesting points. It will be noted that in each series, the infection value of one particular fungus alone exceeded that of the rest of the fungi combined. For example, in Series 1 (corn) the infection value of *Pythium* spp. was 1483, whereas the values of the mycorrhizal fungus, *Asterocystis*, unidentified fungi, and *Helminthosporium* combined amounted to only 1072. Similarly in Series 4 (soybean) the infection value of the predominating fungus, *Thielaviopsis basicola*, was 2695, and that of *Asterocystis* and *Pythium* combined was only 38.

A second significant point is the fact that it is not the same fungus that predominated in the different series. Thus, in the tomato series, the predominating fungus was *Rhizoctonia Solani*, in the soybean series, *Thielaviopsis basicola*, and in the corn series, *Pythium* spp. In both the timothy and red clover series, the infection value of the mycorrhizal fungus was highest. These results suggest that each of the cover crops has apparently favoured the development of a specific fungus, with consequent alterations in pre-existing microbiological equilibria.

In the case of nematodes, differences, both qualitative and quantitative, are no less striking than those recorded for the fungi. In the tomato, soybean and corn series, a total of 191 nematodes was counted in the 3000 mm. of root tissue examined. Most of these represented species which are more usually regarded as saprophytic. In the timothy and red clover series a total of 1692 nematodes were counted in the 2000 mm. of root tissue examined. Almost without exception these were the meadow nematode, *Pratylenchus pratensis* (de Man, 1880) Filipjev, 1936, which represents one of the most ubiquitous and important parasites of the roots of many plants.

ROOTS OF STRAWBERRY PLANTS

Rootlets of the strawberry plants that had completed the three-month period of growth of the second summer season were selected for examination. As in the case of roots of the cover crop plants care was taken to select comparable material. Infection values of the fungi and the numbers and kinds of nematodes were determined by examination of 1000 mm. of root tissue of straw-

berry plants following the respective cover crops. The data are presented in Table III.

Referring to this table, it will be noted that the infection values assigned the fungi vary widely, from zero for plants grown in the steam sterilized soil to 2070 for those grown in the timothy treated soil. In the soybean series, the fungi had total infection value of only 240, which, however, in the manure, untreated (control), corn, and timothy series increases to 828, 1358, 1629, and 2070, respectively. The series in which strawberries follow soybeans is one of the best to illustrate how an appreciable change has been effected in the original microbiological equilibrium of the root rot soil. It will be noted that the mycorrhizal fungus in the roots of the strawberry plants grown in untreated root rot soil has an infection value of 1341. In the roots of the strawberry plants grown in the same soil after the turning under of eight crops of soybeans, the infection value assigned the mycorrhizal fungus was only 216. Referring back to Table II, it will be noted that in the roots of the ninth crop of soybeans themselves, only trace infection by the mycorrhizal fungus was observed; *Thielaviopsis basicola*, however, was so abundant in the roots that it received the high infection value of 2695.

Unlike the fungi, nematodes do not gradually increase in number in the different series but are present in significant numbers in the roots of strawberry plants following timothy only. Almost without exception these belonged to the parasitic species, *Pratylenchus pratensis*.

The correlation pointed out above between soil treatment and degree of infection by fungi and nematodes is not the only one indicated by the data summarized in Table III. An apparent correlation exists also between growth response, as indicated by dry weight of roots of plants and infection by fungi and nematodes, particularly the former. In Series 14 (sterilized soil), where, except for traces of infection, fungi were absent, the roots weighed 33.9 gm., the maximum weight for all series in 1938. As the infection value increased to 240 in Series 4 (soybeans), there was not a proportional four-fold decrease in the weight of the roots. In fact, those of the manure series weighed only 1.6 gm. less than those of the soybean series. By referring back to the description of the macroscopic condition of the roots of these two series, it will be found that although in that season they did not differ appreciably in bulk, they did differ greatly pathologically; those of the manure series showed an almost general discoloration as well as considerable necrosis, whereas those of the soybean series had remained in an extremely healthy condition. In Series 1 (corn), where the infection value is 1629, the decrease in the weight of the roots was again quite marked. Such, too, was the case in Series 7 (timothy) and 15 (control), which make interesting comparison. In the former where the infection value of the fungi was highest, i.e., 2070, and where, too, presumably parasitic nematodes were most numerous, it might be expected that the dry weight of the roots would be lowest. However, the roots in this series weighed more than those of Series 15 (control) where the infection value of the fungi was only 1358. This suggests that in root

rot soil some factor or factors apart altogether from fungi and nematodes must be contributing to the almost complete inhibition in growth and destruction of strawberry roots.

Referring again to Table III it will be noted that the infection value of the phycomycetous mycorrhizal fungus so closely approximates the total infection value assigned all the fungi in each of the different series, that an attempt to evaluate the possible pathological significance of the fungi would resolve itself into giving primary consideration to this organism alone. Such consideration must still remain, however, mostly speculative since, up to the present, no one has succeeded in obtaining sufficient growth of the fungus on artificial media to be able to test its pathogenic capabilities. Consequently, in regard to its significance as a causal agent of root rot, little can be added to opinions already expressed by a number of investigators (9, 12, 3, 4). If the fungus is a parasite, then an interesting point disclosed by these studies is the ease with which its occurrence in root rot soil can be modified by different treatments—additions of corn and timothy apparently contributing to its increase, soybeans on the other hand sharply reducing its incidence. At this point mention should be made of the fact that in the roots of strawberry plants following soybeans, only scattered, incipient infections by this fungus were observed but in the roots of plants following corn and timothy, numerous heavily infected areas showed the fungus in all its stages. If the fungus is not a parasite, then the significance that seemed to attach to it in the correlations mentioned above must be attributed to some other factor or factors. Evidence in support of the latter possibility is not lacking. In many instances it is quite impossible to correlate the presence of the mycorrhizal fungus with discoloured or necrotic tissue. Nor is this true only of the mycorrhizal fungus. In the present studies and in others that have been carried out during the past few years*, some thousands of specimens of diseased roots have been critically examined. Rarely or never, have bacteria been observed *with certainty* in early infections, a fact which caused emphasis to be placed on the fungi in the search for the primary causal agent of the disease. In the majority of cases it has been possible to associate the presence of fungi (other than the mycorrhizal fungus) and nematodes with affected tissues. Cases were repeatedly encountered, however, in which no organism could be found in discoloured and necrotic tissue. In the aggregate the number of these cases reached such proportions that the phenomenon could not be regarded as being without significance. The impression was gained that many roots were injured not directly by actual invasion by parasitic organisms but indirectly by some agency in the soil. The exact nature of this agency remained unknown but circumstantial evidence over a long period of intensive investigation strongly suggested that it was more probably of biotic origin and could not be dissociated from the organisms suspected of being concentrated in root rot soil. The findings reported in the bacteriological section which follows are of considerable interest in this connection.

* Results to be published shortly.

Bacterial Equilibrium of Differently Treated Soils

As was pointed out above, the absence of organisms in discoloured and necrotic tissue of roots of many strawberry plants, strongly suggested the existence of a factor or factors capable of causing the type of injury in question without actual invasion of the roots themselves. Believing that bacteria might in some way be associated with these indirectly harmful effects, there was undertaken a qualitative survey of the general bacteriology of soils capable, on the one hand, of producing healthy plants and, on the other, of inducing symptoms of the disease. Five soils of the cover crop series, each differing significantly in its apparent content of the root rot producing principle were selected for bacteriological study. These were: Series 15, untreated root rot soil; Series 14, steam sterilized root rot soil; Series 16, root rot soil plus barnyard manure; Series 3, root rot soil plus nine crops of red clover; and Series 4, root rot soil plus 12 crops of soybeans.

It is obvious that the interaction between the general soil microflora and higher plants must occur at the root surface. Therefore, primary attention was paid to the isolates obtained from the rhizosphere of roots of the various series, though for purposes of comparison isolations were also made from soil beyond the influence of the roots. The technique employed has been described elsewhere (15, 7, 11).

Apart from any qualitative considerations, it is interesting to note the relative densities of the total bacterial populations that were found in the rhizospheres of plants manifesting varying degrees of root rot injury (Table IV).

Fewer bacteria were present in the rhizospheres of healthy roots than in those of plants affected with the disease. These differences occurred only at

TABLE IV

NUMBERS OF BACTERIA IN THE RHIZOSPHERES OF STRAWBERRY PLANTS IN RELATION TO SOIL TREATMENT AND DEGREE OF INJURY TO THE ROOTS

Series No.	Treatment of root rot soil	Rhizosphere bacteria, millions per gm. root	Bacteria apart from roots, millions per gm. soil	Relative severity of root injury**
15	Untreated (control)	2110	8	+++++
3	Red clover (9)*	1450	7	+++++
16	Barnyard manure (3)	790	10	+++
4	Soybeans (12)	860	10	+
14	Steam sterilized (3)	250	28	—

* Figures in parentheses indicate number of times cover crops were turned under or number of repetitions of other treatments.

** This approximation of injury to roots is based on macroscopic observation supplemented by comparison of dry weights for 1939.

the root surfaces, the soils themselves (except that which was sterilized) supporting approximately equal numbers of bacteria; sterilized soil became repopulated to such an extent that more organisms were present in it than in soils of the other series.

The apparent correlation between bacterial activity at the root surfaces and the severity of injury by root rot indicated the necessity for qualitative analysis of the bacterial flora in each instance. To this end, the isolates from each series were differentiated into nutritional groups according to the method of West and Lochhead (15). Examination of the data thus obtained indicated a striking relationship between the incidence of certain groups of bacteria and the severity of attack by the disease (Table V). Bacteria of Group 3

TABLE V

PERCENTAGE OCCURRENCE OF VARIOUS GROUPS OF BACTERIA IN THE RHIZOSPHERES OF STRAWBERRY PLANTS SHOWING DIFFERENT DEGREES OF ROOT ROT INJURY

Bacterial group No.	Percentage occurrence				
1	0	10.9	0	10.6	18.6
2	29.3	2.2	15.4	0	4.6
3	51.9	26.0	23.0	14.9	2.3
4	0	2.2	0	10.6	0
6	1.3	2.2	0	4.2	2.3
8	6.6	8.7	0	2.1	4.6
10	5.3	23.9	38.4	12.8	20.9
5	0	6.5	0	10.6	7.0
7	2.7	2.2	15.4	4.2	7.0
9	2.7	15.2	7.7	29.7	32.4
	5.4	23.9	23.1	44.5	46.4
Degree of injury	++++++ ++++	+++++ ++	+++	+	—
Series	Untreated soil	Red clover	Manure	Soybean	Sterilized soil

accounted for 51.9% of all organisms present at the root surface of the badly diseased plants in untreated root rot soil. These organisms became less and less abundant as the roots in the remaining four series showed greater and greater freedom from disease until, in sterilized soil, only 2.3% of the rhizosphere population was of this type. On the other hand, Groups 5, 7, and 9, which are known to be specifically favoured at the root surface of certain healthy plants (13, 14) comprised 46.4% of the bacteria in the rhizosphere of the healthiest roots, and only 5.4% in that of plants most severely affected with the disease. It is evident that the normally occurring rhizosphere types (Groups 5, 7, 9) which decrease in number as the disease becomes more severe, are at the same time being replaced by organisms of Group 3 (Fig. 1). This equilibrium has been conveniently expressed numerically as the "Bacterial Balance Index" (15). The remaining groups of bacteria were less well represented and do not appear to be correlated with the incidence of the disease.

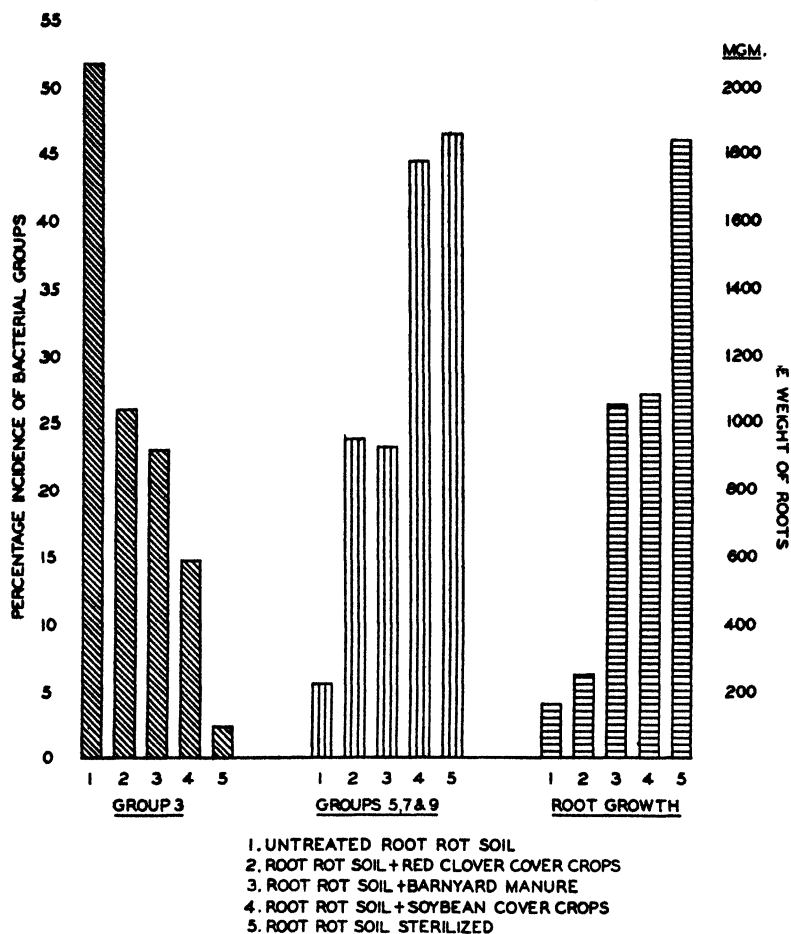


FIG. 1. Correlation between occurrence of harmful (Group 3) and normally occurring rhizosphere types (Groups 5, 7, 9) of soil bacteria and severity of root rot as indicated by dry weights of surviving roots.

The striking relationship existing between the kinds of bacteria occurring on strawberry roots and the severity of the disease is clearly shown by a comparison of the Bacterial Balance Indices of rhizosphere soils from each of the five series of plants (Table VI).

TABLE VI

THE "BACTERIAL BALANCE INDEX" OF SOIL ADJACENT TO AND APART FROM THE ROOT SURFACES OF STRAWBERRY PLANTS SHOWING DIFFERENT DEGREES OF ROOT ROT INJURY

Series No.	Treatment of root rot soil	Relative severity of the disease	Bacterial Balance Index	
			Rhizosphere soil	Soil apart from roots
15	Untreated	+++++	-46	10
3	Red clover	+++++	-2	13
16	Barnyard manure	+++	0	19
4	Soybean	+	30	32
14	Steam sterilized	-	44	35

Corresponding values calculated from qualitative data for soils apart from the zone of influence of the root are also shown. It is apparent from these values that very marked differences exist in the microbiological equilibria of the five soils, and that the lower the Bacterial Balance Index falls, the more intensified the root rot factor becomes. The differences that occur between the five control soils (i.e., apart from the roots) are paralleled to an accentuated degree in the rhizospheres of plants grown in those soils. It is interesting to note that the incorporation of soybean cover crops into root rot soil causes a shift in the bacterial balance in the same direction as that produced by sterilization, the change being accompanied by a decrease in the root rot factor. The other leguminous crop, red clover, causes relatively little change in the equilibrium and brings about correspondingly little improvement in the condition of the roots.

As demonstrated previously (14, 15) certain plants, including strawberries, when growing in a "healthy" soil, exert a modifying influence on the soil microflora such that the bacteria requiring amino acids or growth substances are favoured, whereas other organisms are reduced in numbers. This is referred to as the "normal" or "positive rhizosphere effect", and corresponds to an increase in the Bacterial Balance Index of the rhizosphere soil over that of the surrounding unaffected soil. As shown in Table VI the healthy plants growing in sterilized soil exerted the usual positive rhizosphere effect. However, when the soil balance shifts in favour of the types particularly associated with root rot (Group 3), the normal effect of the plant on the microflora on its root surface is modified. At first as in the soybean treated soil, the rhizosphere influence is merely neutralized, and then in definitely "disease" soils, it swings strongly in the reverse direction. The reason for this reversal is not yet known. This abnormal change in which the unfavourable Group 3 bacteria are selectively stimulated at the root surface is referred to as the "negative rhizosphere effect". Apparently if Premier strawberry plants are grown in a soil with a low Bacterial Balance Index, the normal beneficial influence of the plant is overcome and the bacterial equilibrium at the root surface becomes even more unfavourable than throughout the rest of the soil.

Some indication of the manner in which Group 3 bacteria may exert a harmful effect on strawberry roots was obtained experimentally as follows. Selected portions of clean white roots washed in distilled water and cut into quarter-inch sections were placed in each of three Petri plates. To one lot was added tap water, to the second the mixed liquid cultures of approximately 50 organisms isolated from the rhizosphere of healthy plants growing in sterilized soil and to the third the mixed cultures of organisms isolated from badly rotted roots grown in the untreated root rot soil. After 24 hr. at room temperature the roots immersed in the bacterial suspension from diseased plants were becoming definitely discoloured, and by 48 hr. they were deep brown to black in colour and were reduced to a soft, flabby consistency. Even after six days, the root sections in the other bacterial suspension were clean and white with no more discoloration or loss of firmness than those in

p water. No attempt had been made to exclude accidental air contaminants in this work. Further investigation showed that the deleterious effects of the bacterial isolates from root rot plants were the result of the activity of certain components of the Group 3 organisms. A series of preliminary infection tests indicated that such bacteria are capable of causing stunting and discoloration of the roots of strawberry seedlings. These observations together with the data showing correlation between numbers of Group 3 bacteria and the severity of attack by the disease would seem to indicate that the bacteriological balance of the soil is a factor that can by no means be eliminated from a consideration of the strawberry root rot complex.

Discussion

Garrett (2, p. 26) has recently pointed out that "the soil population is a dynamic biological equilibrium". The results of the present studies add emphasis to the truth of this statement. They have shown that populations of organisms including representatives of certain groups of fungi, nematodes, and bacteria, which have become established in a certain soil and which, as a result, have rendered that soil inimical to the healthy growth of varieties of the cultivated strawberry, are amenable to change. The new microbiological equilibria which have become established following the various treatments are still dynamic. In some cases, as for example in timothy and red clover treated root rot soil, the new components which are apparently dominant in the altered equilibria, still exert a deleterious effect on the strawberry, whereas in others, as in soybean treated soil, the effect is just as markedly beneficial.

In the present studies associative phenomena of the various types of organisms concerned have not been studied in sufficient detail to aid in a clearer understanding of the principles and the mechanism of associative effects. Thus, it is not known by what process the potentially parasitic phycomycetoid type of mycorrhizal fungus, the definitely parasitic nematode *Pratylenchus pratensis*, and the composite group of "harmful" bacteria, practically disappear from soybean treated root rot soil, whereas in the same soil under similar treatment *Thielaviopsis basicola* so greatly increases. Whether in this instance, such changes are due to competition and antagonism alone, or whether they are related to a changing chemistry of the soil, is not known at the present time.

So far as known detailed qualitative studies of the rhizosphere flora of strawberry roots have not been reported heretofore. Such studies, in the present investigations have shown that the incidence of certain groups of bacteria are definitely correlated with the intensity of the root rot factor. Thus it becomes imperative to broaden the concept of the microbiological factor, in relation to strawberry root rot. Heretofore, emphasis has been placed almost exclusively on the fungus component. Although little doubt exists as to the parasitic capabilities of certain fungi and nematodes, never-

theless the clear-cut bacteriological correlations observed indicate that certain bacteria must be equally important components of the microbiological complex. The extremely high concentration of "abnormal" types of bacteria at the root surface of diseased plants suggests that they may well be the primary cause of much discoloration that occurs on the diseased roots, as well as contribute to the general susceptibility of the plant to fungus attack.

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THE MICROBIOLOGICAL BALANCE OF STRAWBERRY ROOT ROT SOIL AS RELATED TO THE RHIZOSPHERE AND DECOMPOSITION EFFECTS OF CERTAIN COVER CROPS¹

BY P. M. WEST² AND A. A. HILDEBRAND³

Abstract

Soybean and red clover, grown as cover crops and incorporated into strawberry root rot soil, showed a marked difference in ability to control the disease on variety Premier. Soybean caused a striking reduction in the incidence of root rot and a drastic shift in the bacterial equilibrium of the soil. Red clover had little effect on the severity of the disease or the general microflora of the soil.

A study of "rhizosphere effects" reveals that the characteristic differences between the resultant bacterial equilibrium of the soils in which the two leguminous plants were grown, could not be attributed to influences exerted by the latter in the living state. However, the bacterial types favoured during decomposition in experimental cultures of tissues of red clover and of soybean, each inoculated with root rot soil, were identical with those isolated from root rot soil with which red clover and soybean, respectively, had been incorporated. In contrast to the putrefactive decomposition of red clover, soybeans apparently underwent a carbohydrate breakdown that could be reproduced essentially in culture by the substitution of glucose for soybean tissues. Beneficial changes in the bacteriology of actual root rot soils could be induced by the decomposition of pure carbohydrate in place of soybean. The favourable alteration in the bacterial equilibrium was accompanied by a corresponding modification of the fungous flora such that potentially pathogenic forms were replaced by presumably innocuous ones. These carbohydrate treated soils were capable of producing strawberry plants with well developed healthy root systems. The ability of soybean to control strawberry root rot therefore seems to depend primarily on a carbohydrate type of breakdown in diseased soil, causing a highly favourable shift in the microbiological equilibrium. The decomposition of red clover, on the other hand, did not under the same conditions induce these salutary effects.

Introduction

Previously reported investigations by the authors of the present paper (7), concerning the possibility of biological control of root rot of strawberry, showed that under experimental conditions the severity of the disease could be reduced by the repeated turning under of certain cover crops. Soybeans were found capable of exerting a corrective influence second only to that following sterilization by steam. Incorporation of this plant into naturally infected root rot soil resulted in a striking reduction in the incidence of the disease, this highly salutary effect being accompanied by a marked alteration in the bacterial equilibrium of the soil. Red clover, on the other hand, exerted the least beneficial effect of all the cover crops tried and induced only minor qualitative changes in the population of the root rot soil. Because of the striking contrast in the influence of these two leguminous plants, they were selected for further study which, it was hoped, might suggest fundamental reasons underlying the difference in effectiveness of the various cover crops in modifying the severity of the disease.

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Experimental

The Influence of the Living Plant

It is apparent that the cover crop plants under consideration might exert their characteristic effects on the microbiological population of the root rot soil either while in the living state, or while decomposing in the soil, or both. Since it is well known that growing plants are capable of modifying the bacterial flora in the immediate vicinity of the roots (9, 10, 13, 14, 15, 19, 25), this phase of the problem was considered first. Soybean and red clover were grown from seed in pots of the highly "potent" root rot soil used in earlier investigations (7), and after six weeks, representative bacteria were isolated from the rhizospheres by methods previously outlined (7). The isolates were studied qualitatively by the procedure of West and Lochhead (26) and the bacterial equilibrium existing at the root surfaces of the two leguminous plants was expressed as the Bacterial Balance Index. A summary of these data together with corresponding figures for strawberry seedlings of Premier parentage grown for four weeks in the same soil are shown in Table I.

TABLE I

THE BACTERIAL BALANCE IN THE RHIZOSPHERES OF SOYBEAN, RED CLOVER, AND STRAWBERRY SEEDLINGS GROWN IN ROOT ROT SOIL

Soil	Percentage incidence of bacterial groups		Bacterial Balance Index	Rhizosphere effect
	Group 3	Groups 5, 7, 9		
Root rot soil control	7	17	+10	—
Strawberry* rhizosphere	48	20	-28	Negative
Soybean rhizosphere	42	15	-27	Negative
Red clover rhizosphere	13	32	+19	Positive

*Plants grown from seed originating from fruit of Premier variety.

In conformity with earlier findings (7), the strawberry seedlings became severely affected with root rot and exerted a "negative rhizosphere effect". Although the occurrence of favourable bacteria (Groups 5, 7, and 9) in the rhizospheres of these plants was not appreciably altered, the unfavourable types (Group 3) characteristically associated with the root rot condition were tremendously increased, thereby causing a sharp fall in the Bacterial Balance Index. Contrary to expectations, the bacterial equilibrium in the rhizosphere of the healthy soybean roots was almost identical with that of the roots of the strawberry seedlings. In the rhizosphere of red clover, however, the bacteria requiring specific nutritive substances (24, 25, 26) were selectively stimulated, and a typical normal or positive rhizosphere effect was the result.

The final outcome of the soybean treatment of root rot soil is a pronounced increase in the Bacterial Balance Index of the soil in general, caused by the

reduction in numbers of unfavourable bacteria (Group 3) and an increase in the types normally associated with freedom from disease (Groups 5, 7, and 9). It is clear that if the influence of the living plant, i.e., the rhizosphere effect, were the important factor concerned in shifting the bacterial balance of the root rot soil, then red clover would cause a rise in the Bacterial Balance Index whereas soybean would exert no such beneficial effect. Since the reverse is actually the case, the conclusion must be drawn that the influence of the growing plant on the bacterial balance of the soil is a relatively minor one and does not determine the nature of the extensive bacteriological changes that occur when the legumes are grown and incorporated into root rot soil. Apparently more potent and significant effects are exerted by these cover crops in some other way.

The Influence of the Decomposing Plant

When it became apparent that the difference in the abilities of the two leguminous cover crops to alter the microbiological balance of root rot soils was not associated with the actual growth of the plants, attention was turned to a study of the bacteriological aspects of their decomposition. Leaves and stems of clover and soybean grown under identical conditions in root rot soil for six weeks were finely chopped, 2.5 gm. of each placed in separate six-ounce culture bottles, 50 ml. of tap water added, and the mixture inoculated with 0.5 gm. of root rot soil, the Bacterial Balance Index of which was known. After two weeks' incubation at 28° C., the decomposing tissues exhibited the characteristics summarized in Table II.

TABLE II
GENERAL CHARACTERISTICS OF DECOMPOSITION OF SOYBEAN AND RED CLOVER
AFTER 14 DAYS

Plant	pH	Millions of bacteria per ml.	Odour	Characteristics of breakdown
Soybean	4.8	1.3	Clean, acid, aromatic	Retention of green colour
Red clover	6.6	147.0	Foul, putrefactive	Colour change to dark brown—disintegration almost complete

It is evident that the breakdown of the two leguminous plants under identical conditions was strikingly different. The sharp contrast between bacterial numbers alone is suggestive of qualitative variations in the two. Apparently the soybean underwent a predominantly carbohydrate fermentation with the production of volatile organic acids, whereas the decomposition of red clover was chiefly putrefactive.

Isolations were made from the decomposing plant tissues by plating on soil-extract agar (10, 17) and representative organisms from each culture

were studied qualitatively to determine what bacterial types added in the original inoculum were favoured during the decomposition of the two crops. The results are summarized in Table III. For purposes of comparison, other data are included to show the changes in the bacterial equilibrium that followed incorporation of the two cover crops into root rot soil (7).

TABLE III

MODIFICATION OF THE BACTERIAL EQUILIBRIUM BY DECOMPOSITION OF RED CLOVER AND SOYBEAN IN ROOT ROT SOIL AND IN ARTIFICIAL CULTURE

—	Percentage incidence of bacterial groups		Bacterial Balance Index
	Group 3	Groups 5, 7, 9	
Untreated root rot soil	7	17	+10
Root rot soil following incorporation of nine crops of red clover	12	25	+13
Decomposing red clover culture	23	30	+ 7
Root rot soil following incorporation of 12 crops of soybeans	3	38	+35
Decomposing soybean culture	0	42	+42

It will be observed that after several crops of red clover were added to root rot soil, there was a small increase in both favourable and unfavourable bacterial groups, resulting in little net change either in the Bacterial Balance Index or in the improvement of the soil for strawberries. An exactly parallel but more pronounced effect on the bacterial population was found to result from the decomposition of red clover tissues when inoculated with root rot soil.

The incorporation of soybean cover crops into root rot soil resulted in an inhibition of unfavourable bacteria and a pronounced stimulation of favourable types, accompanied by a striking improvement in the Bacterial Balance Index and in the productivity of the soil. Again, an exactly parallel and somewhat intensified effect on the bacterial equilibrium was found to be characteristic of the decomposition of soybean tissues when inoculated with root rot soil.

These data suggest that the difference in ability of soybean and red clover cover crops to alter the microbiological balance of root rot soils depends primarily on differences in the chemical constitution of the two plants. When the plants are added to the soil these differences are reflected in the nature of their decomposition—carbohydrate fermentation in the one and putrefaction in the other. The bacteria concerned in the former process are the types associated with "healthy" strawberry soils, those concerned in the putrefactive breakdown are not. Thus the incorporation of soybean cover

Crops into root rot soils causes a very favourable shift in the bacterial equilibrium, whereas red clover lacks this capacity. The situation is complicated by the fact that variations occur in the chemical composition of a crop depending on its stage of growth (8, 11, 18, 21, 22, 23) and a green manure might therefore be expected to exert a greater influence on the microbiological equilibrium of the soil at one stage than at another. However, previous results (7) as well as those described above, indicate that between species of plants of the same age, grown under identical conditions, there are inherent differences in composition which are highly significant in determining the type of micro-organisms concerned in their breakdown in the soil.

The above findings suggested that if the stimulating effect of decomposing soybean on the development of favourable bacteria depends on the carbohydrate type of decomposition which the tissues undergo in root rot soil, then similar changes might be induced by the substitution of a pure carbohydrate for the plant itself. With this in mind, an amount of commercial glucose equal to the weight of soybean tissue previously used, was placed in a culture bottle, 0.1% yeast extract was added to provide the necessary growth substances for the favourable bacteria, and water and root rot soil were then added as before. After two weeks' incubation the culture emitted a clean, acid aroma and its pH was 4.3. Superficially at least the breakdown of soybean was very similar to the fermentation of glucose.

A qualitative study of representative organisms isolated from the culture revealed the close parallelism existing between the decomposition of soybean tissue and the fermentation of glucose. In the latter the proportion of unfavourable organisms was reduced to 3%, while numbers of favourable bacteria rose to 65%. Thus the breakdown of pure carbohydrate raised the Bacterial Balance Index to +62, even higher than did the decomposing plant itself (cf. Table III). The successful replacement of soybean tissues with glucose in these decomposition experiments was of especial interest, because there was suggested the possibility of a simple means of experimentally altering the microbiological equilibrium of diseased soils and of studying the effects of such changes on the incidence and severity of strawberry root rot.

The Significance of Carbohydrate Breakdown

The fact that a highly favourable shift in the bacterial equilibrium of strawberry root rot soil could be brought about in culture flasks by the decomposition of either soybean or glucose, suggested treating actual root rot soil with a carbohydrate to determine whether or not the root rot factor could be controlled as effectively by this means as by the incorporation of soybean cover crops. The highly "potent" root rot soil used in earlier investigations (7) was selected for trial. To this soil, commercial glucose was added at the rate of 25 gm. per 3-in. pot of soil. Another source of organic carbon (acetic acid) which was considered likely to undergo decomposition similar to that of glucose was added to other 3-in. pots of root rot soil until the pH of the latter reached 4.5. Care was taken to add only

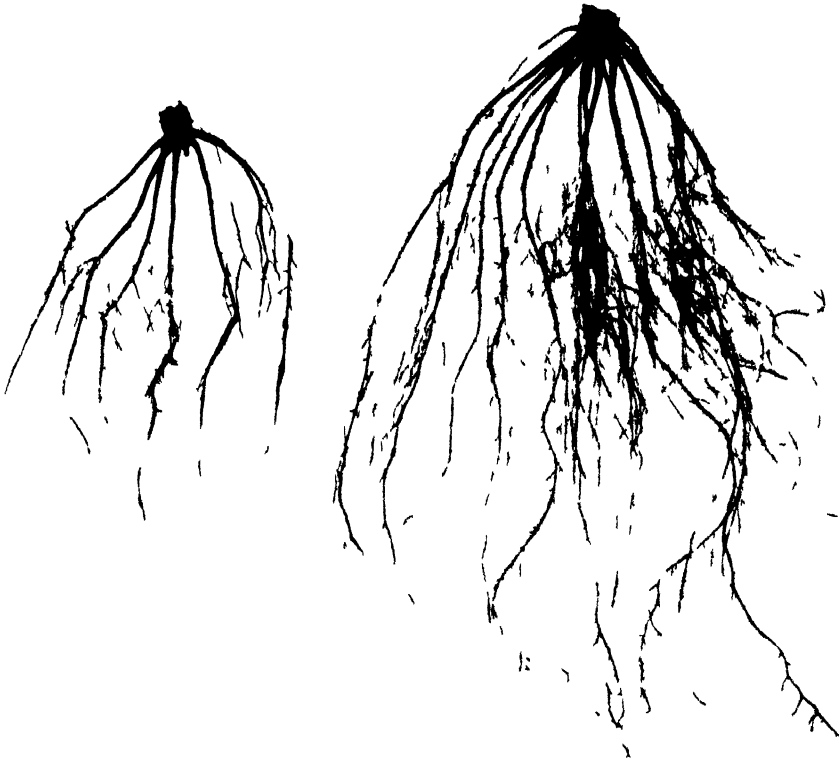
sufficient acetic acid to provide a satisfactory substrate for bacterial activity but not enough to exert any sterilizing effect. Both treated soils and untreated control root rot soil were kept in a moistened condition at room temperature for four weeks. During this time a vigorous carbohydrate fermentation took place in the glucose treated soil, as evidenced by a definite aroma of volatile organic acids and a drop in pH from 6.8 to 6.2 after six days. After four weeks, the reaction of both treated soils had returned to the original pH of 6.8, indicating that oxidation of all the organic acids had occurred. At that stage, four healthy strawberry seedlings (Premier parentage) of uniform size, were planted in the soils treated with glucose and acetic acid and in the untreated soil. The seedlings were allowed to grow in the three soils for 30 days. As had been observed on other occasions, seedlings grown in the untreated soil made little progress—rather, it was found that they had retrogressed and their roots, originally white and healthy, had become almost completely discoloured and necrotic. In marked contrast, the seedlings in the treated soils grew very rapidly, developing into vigorous young plants, with healthy and extensive root systems. The weights of the roots of the four seedlings from each series are shown in Table IV.

TABLE IV
GROWTH OF STRAWBERRY SEEDLINGS IN ROOT ROT SOIL
AND IN THE SAME SOIL AFTER DECOMPOSITION OF
GLUCOSE AND ACETIC ACID

Soil treatment	Weight of roots of four seedlings, mg
Root rot soil untreated	50
Root rot soil + glucose	395
Root rot soil + acetic acid	510

Ten weeks from the date of the original treatments, Premier runners were struck in the non-treated and in the glucose treated soils in which the seedlings had already been grown. At the end of 30 days the plants in the non-treated soil were noticeably stunted and their roots not only lacked bulk but showed numerous, extensive reddish-brown to black lesions. On the other hand, plants grown in glucose treated soil made rapid development and their roots were bulky, white, and healthy (Plate I). These findings made it evident that not only could carbohydrate breakdown completely and effectually replace that of soybean in shifting the bacterial flora in soil cultures, but could also induce, in presumably the same manner, all the microbiological changes attendant upon the conversion of a "potent" root rot soil into a decidedly more healthy one. Moreover, the beneficial effect on the runner plants was in no way impaired by the growth of the seedlings that preceded them, thus indicating a residual effect of the glucose treatment.

PLATE I



Roots of Premier runner plants grown for 30 days in untreated (left) and glucose treated (right) root rot soil, showing how effectively the decomposition of the carbohydrate has modified the severity of the disease

Qualitative study of the bacterial flora in the three soils after the removal of the seedlings yielded the results shown in Table V. These make interesting comparison with those shown in Table III. The bacterial equilibrium of the root rot soil to which either glucose or acetic acid was added, shifted in the same way but to an even greater degree than soil that received soybean cover crops. Group 3 bacteria were reduced in numbers, whereas the incidence of Groups 5, 7, and 9 was enormously increased, causing a decidedly favourable upswing in the Bacterial Balance Index. Since carbohydrate fermentation appears to be the fundamental basis for these changes, it is only to be expected that the pure compounds would have a greater effect than the soybean tissues themselves. It will also be noted that the change in the bacterial equilibrium that occurred during the decomposition of both soybean and glucose in artificial culture was more pronounced than in soil; this apparently was due to the greater buffering effect in the soil.

TABLE V

COMPARISON OF THE BREAKDOWN OF SOYBEAN TISSUES WITH THAT OF GLUCOSE AND ACETIC ACID IN RELATION TO THE BACTERIAL BALANCE OF STRAWBERRY ROOT ROT SOILS

Soil treatment	Percentage incidence of bacterial groups		Bacterial Balance Index
	Group 3	Groups 5, 7, 9	
Root rot soil untreated	7	17	+10
Root rot soil after decomposition of soybean cover crops	3	38	+35
Root rot soil after decomposition of glucose	0	45	+45
Root rot soil after decomposition of acetic acid	3	47	+44

Since all micro-organisms of the soil are biologically inter-related, it might reasonably be expected that environmental changes that are great enough to alter materially the bacterial equilibrium, probably at the same time would be capable of exerting equally profound influences on the fungous flora. To determine the effect of the glucose and acetic acid treatments of root rot soil on the fungi, a rhizosphere study of the seedlings from the three series was made. After the roots were vigorously shaken in sterile water to remove bacteria for qualitative analysis, they were further washed in running water for 1 hr. From each series, 51 representative sections of root, about one quarter inch in length, were "planted" on the surface of acidified and non-acidified potato dextrose agar plates, three per plate. The development of fungi from these platings was followed microscopically for several days, identification being made either directly from the plates or from cultured hyphal tips. As a result of the thorough washing, the fungi obtained from the rootlets represented either those from within the root tissues or those in intimate contact with the root surface.

The various fungi obtained from the obviously diseased roots of the Premier seedlings grown in the untreated soil included representatives of genera that are almost invariably isolated from plants affected with root rot, and to which also different investigators have attributed pathogenic capability (Table VI). On the other hand the relatively healthy roots of the seedlings grown in soil in which glucose or acetic acid had decomposed, gave a quite different picture. Very striking is the reduction of *Cylindrocarpon* from 29.1% of all fungi at the root surface of plants in untreated soil,

TABLE VI

THE IDENTITY AND PERCENTAGE OCCURRENCE OF FUNGI ON ROOTS OF STRAWBERRY SEEDLINGS AS INFLUENCED BY THE DECOMPOSITION OF GLUCOSE OR ACETIC ACID IN ROOT ROT SOIL

Root rot soil untreated			Root rot soil + glucose			Root rot soil + acetic acid		
Fungi			Fungi			Fungi		
Species	Total number	%	Species	Total number	%	Species	Total number	%
<i>Actinomycetes</i>	1	1.8	<i>Actinomycetes</i>	6	16.6	<i>Actinomycetes</i>	2	3.9
<i>Cephalosporium</i>	1	1.8	Fungus A	1	2.7	Fungus C	1	1.9
<i>Cylindrocarpon</i> spp	16	29.1	Fungus B	1	2.7	Fungus D	1	1.9
<i>Fusarium</i> spp	24	43.6	<i>Fusarium</i> spp	9	25.0	<i>Fusarium</i> spp	13	25.5
<i>Gliocladium</i>	1	1.8	<i>Hormodendrum</i>	2	5.5	<i>Mucor</i>	4	7.8
Orchid <i>Rhizoctonia</i>	1	1.8	<i>Mucor</i>	2	5.5	<i>Penicillium</i> spp	26	51.0
<i>Penicillium</i> spp	6	10.9	<i>Penicillium</i> spp	6	16.6	<i>Trichoderma</i>	4	7.8
<i>Phoma</i>	1	1.8	<i>Phoma</i>	1	2.7			
<i>Trichoderma</i>	3	5.5	<i>Trichoderma</i>	1	2.7			
<i>Verticillium</i>	1	1.8	<i>Verticillium</i>	1	2.7			
			Yeast	6	16.6			
	55			36			51	

to zero in the other two root systems (Fig. 1). Berkeley and Lauder-Thomson (2) and Richards and McKay (12) have ascribed importance to *Cylindrocarpon* species as primary parasites capable of inducing a characteristic type of root rot. In addition, the closely allied genus *Fusarium*, also of considerable pathological significance (2, 12, 20) was reduced by the carbohydrate treatment from 43.6 to 25.0%. The endophytic *Rhizoctonia* of the type found commonly in the Orchidaceae and frequently associated with root rot of strawberry (4, 6, 20) was isolated from the seedlings grown in the untreated soil but not from those in the treated soil. The reduction in percentage occurrence of the above mentioned fungi, or, in some cases, their complete disappearance, was necessarily accompanied by an increase of other types. The latter for the most part were considered to be "harmless" forms. The incidence of *Penicillium*, for example, increased from 10.9% in the untreated soil to 51.0% in the acetic acid treated soil. Others occurring more abundantly or exclusively in the treated soils included actinomycetes, yeasts, *Mucor*, and *Hormodendrum*.

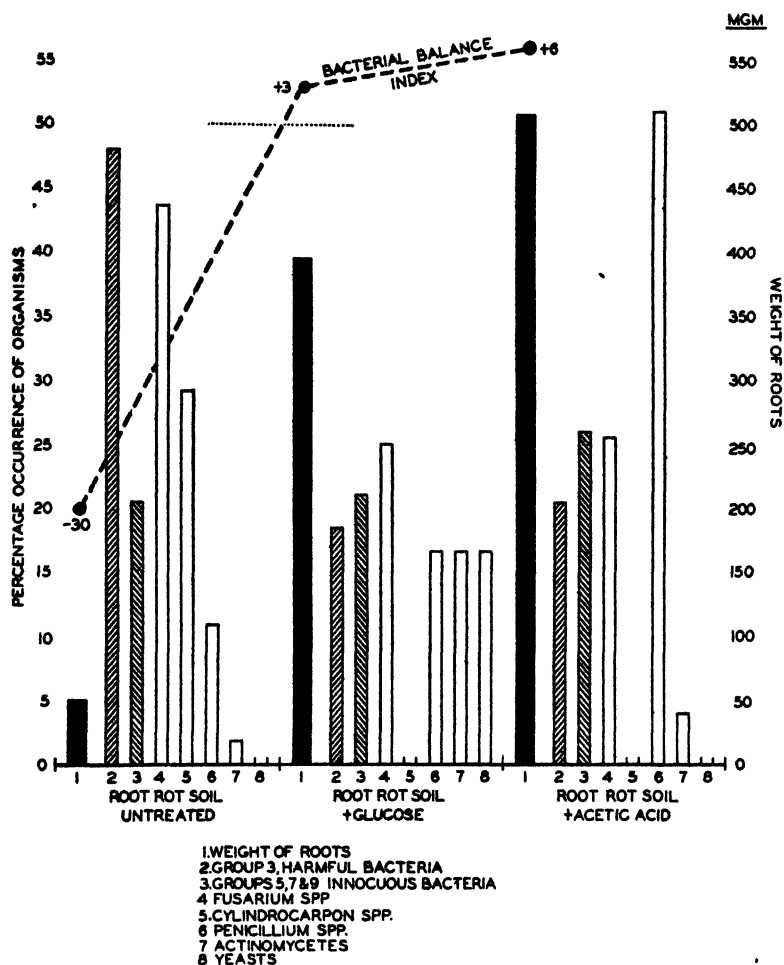


FIG. 1. Influence of carbohydrate decomposition in root rot soil on the bacterial and fungal populations in the rhizospheres of Premier seedlings.

In addition to the qualitative differences pointed out above, it will be noted (Table VI) that there are also quantitative differences. For example, whereas a total of 55 fungous isolates was obtained from the roots of the seedlings grown in the untreated soil, only 36 developed from those grown in the glucose treated soil.

The changes in the bacterial equilibria of the rhizospheres following carbohydrate treatment are similar to those already described for the soil apart from the roots. The Bacterial Balance Index was significantly increased, owing primarily to a marked reduction of Group 3 unfavourable bacteria (Fig. 1). Total bacterial counts in the rhizosphere were not widely divergent (248, 223, and 407 millions per gm. of root system for untreated, acetic acid, and glucose treated soils, respectively) in contrast with the differences in

density of population of the soil apart from the roots (11,100 and 370 millions per gm.).

The above results show, then, that the beneficial change in the bacteriology of root rot soil that followed the breakdown of carbohydrate was accompanied by correspondingly favourable modification of the fungous flora, some of the inter-relationships being illustrated in Fig. 1. Apparently the ability of soybean to modify so effectually strawberry root rot depends primarily on the carbohydrate type of decomposition which the plant undergoes in diseased soil and which produces a highly favourable shift in the general microbiological balance.

Discussion

As more and more data in connection with strawberry root rot are accumulated, the more the complexity of the disease is realized. To date, its cause has been investigated more intensively from the microbiological than from other avenues of approach. The microbiological factor, alone, is a most complicated one as the following brief consideration of a few of the papers that have appeared on the subject during the past few years will show. In 1931, Strong and Strong (16), in demonstrating in pot experiments the etiological relationship of the imperfect stage of *two* Ascomycetes, proved the infectious nature of the disease for the first time. In 1934, Berkeley and Lauder-Thomson (2) demonstrated, also in pot experiments that *five* different fungi (two of them probably identical with those of Strong and Strong) were capable of parasitizing strawberry roots and of producing root rots the symptoms of which were "very much alike, if not identical". Also, in 1934, Truscott (20) found that representatives of *seven* different genera of fungi possess parasitic capability. In the same year, Hildebrand (4) mentioned that nematodes had been encountered in association with diseased strawberry roots in numbers and frequency sufficient to suggest a possible causal relationship to the disease. Recently, the present authors (7) have demonstrated that in addition to a fungous factor and other biotic factors that may be involved, there is also a bacterial factor which can by no means be eliminated from a careful consideration of the strawberry root rot complex.

In any given soil these various organisms are in equilibrium with their environments and with each other and thus no single component of this inter-related system may be disturbed without affecting the whole. This point is illustrated by the decomposition studies described in the present paper, which demonstrate not only the ease with which the microbiological balance may be shifted but also the interaction that occurs between two important groups of components of the complex. Thus, in comparing the bacteriological and mycological data (Fig. 1), it becomes clear that the beneficial effects of the carbohydrate type of organic decomposition in soil as related to strawberry root rot, cannot be defined in terms of what happens to any one species of fungus or any one group of bacteria. Rather, it seems that it is the sum of all the microbiological changes that must be taken into

account in describing the influence of the soil treatments on the root rot picture.

An interesting observation concerning the corrective effect of carbohydrate decomposition on the microflora of root rot soils is the beneficial nature of the qualitative changes in both bacteria and fungi. The bacterial balance was altered in such a way that those organisms consistently associated with strawberry root rot were reduced, and harmless forms increased. Genera of potentially pathogenic fungi, which thrived together with unfavourable groups of bacteria, were replaced by other innocuous types at the same time as the bacterial equilibrium improved.

That these microbiological changes which occur as a result of carbohydrate breakdown in strawberry root rot soils may not necessarily prove beneficial in other root rots is suggested by the work of Eisenmenger (3). His observations indicated that carbohydrate decomposition increased the severity of brown root rot disease of tobacco. This may be correlated with the observations of Berkeley and Koch (1) who warned that previous crops of soybeans in certain types of soil might accentuate brown root rot of tobacco grown later. In the light of the concepts mentioned above, it would seem that the problem of control of the disease is probably not concerned with the elimination of any single organism but rather with effecting relatively profound changes in the general microbiology of root rot soil—such changes as are apparently induced by the incorporation of the soybean or certain carbohydrates as sources of energy. In general, it appears that the biological oxidation of carbohydrate materials in strawberry root rot soil reduces the severity of the disease by bringing about a marked modification of the bacterial and fungal floras of the soil in such a way that potentially "harmful" organisms are greatly reduced and a relatively more favourable microbiological balance becomes established.

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EFFECTS OF PHYTOHORMONE, POTASSIUM NITRATE, AND ETHYL MERCURIC BROMIDE ON THE GERMINATION AND EARLY GROWTH OF WHEAT¹

BY N. H. GRACE²

Abstract

Marquis wheat treated with a series of talc dusts containing indolylacetic acid, potassium naphthylacetate, potassium nitrate, and ethyl mercuric bromide was grown in soil in the greenhouse. Potassium naphthylacetate increased the final germination count 2.6% while indolylacetic treatments failed to affect germination or early growth. Neither of these growth stimulating chemicals interacted with potassium nitrate or ethyl mercuric bromide. Ethyl mercuric bromide retarded the germination rate but increased the final germination count. Potassium nitrate in conjunction with the organic mercurial disinfectant reduced final germination and the air-dry weight of young plants. On the average, dust treatments reduced the rate of germination but increased the air-dry weight of stems.

It has been shown that there are conditions under which phytohormone treatments of cereal and other seeds influence germination and early growth and even the final yields (1, 4, 6, 7, 9-11, 13, 15, 18-20, 23), though lack of effect, or injury from overdosage, have been noted more frequently (3, 4, 8, 10, 16, 17, 21, 22, 24, 25). Small amounts of certain nutrient salts applied to the seed in a dust disinfectant appeared to have some effects on early growth of wheat (12). In consequence, it was considered of interest to investigate the effects of combinations of phytohormone and nutrient salts with seed disinfectants on germination and early growth of wheat in the greenhouse. This communication describes the results of experiments in which wheat seed was treated with growth stimulating chemicals, potassium nitrate, and ethyl mercuric bromide in a talc carrier.

Experimental

The experiments involved treatments with dusts containing indolylacetic acid and the potassium salt of naphthylacetic acid. Each of these chemicals were used separately at concentrations of 0, 0.192, 0.576, 0.96, and 1.92 (parts of chemical in 100 parts of the talc mixture by weight) and in combination with 0 and 3.71% potassium nitrate and 0 and 5% ethyl mercuric bromide (5). These percentages of phytohormone chemical in talc when applied to seed at the rate of one-half ounce per bushel effected treatments of 0, 1, 3, 5, and 10 parts of chemical to a million parts of seed by weight. The entire series for each phytohormone chemical involved the preparation of 20 dusts. Master dusts were prepared first and by their mixture and the addition of talc, the various concentrations and combinations.

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Marquis wheat seed was treated with these dusts at the rate of one-half ounce of dust per bushel of seed in the manner previously described (12). Seed was planted, between 24 and 48 hr. after application of the dust, in small cardboard flats containing unsterilized soil, 50 seeds to each flat. The investigation involved two separate experiments. Experiment 1 comprised treatments with indolylacetic acid and Experiment 2, those with potassium naphthylacetate. Plants in the former were started February 15, 1940, and removed approximately six weeks later, whereas those of the latter were planted April 5 and the plants removed five weeks thereafter. Each experiment involved 10 randomized replicate blocks of the 20 dust treatments and, in addition, an untreated control flat to each block. Thus each experiment required 210 flats with a total of 10,500 seeds.

Temperature in the greenhouse room was maintained around 50° F. during the night and ranged between 55° and 60° F. in the daytime. Although temperature conditions were similar for the two experiments there was greater intensity and duration of the light for the second experiment owing to the advancing season.

Daily germination counts were made as soon as emergence commenced and the germination rate was computed by the method of Bartlett (2). The plants were washed to remove the soil and the air-dry weight of entire plants, as well as of stems and roots, was determined (11-13). All the data were subjected to analyses of variance.

Results

In Table I are given results of the analyses of variance of the data. Indolylacetic acid treatment failed to produce any significant effects whereas potassium naphthylacetate treatment affected the final germination count. Phytohormones failed to interact with the other chemicals. Organic mercury treatment had a marked effect on the germination rate in both experiments and on the final germination count in one. Potassium nitrate treatment interacted with organic mercury.

Experiment 1—Treatments with indolylacetic acid, potassium nitrate, and ethyl mercuric bromide

Organic mercury treatment effected a decrease of 2.4% in the germination rate, the value being 68.3% in its absence and 65.9% in its presence. Conversely, the final germination count was increased from 89.0 to 92.2%. The air-dry weight of plants was unaffected.

Experiment 2—Treatments with potassium naphthylacetate, potassium nitrate, and ethyl mercuric bromide

The growth stimulating chemical increased the final germination count, on the average, by 2.6%. In its absence the value was 88.4% and the average for its four concentrations, which did not differ significantly among themselves, was 91.0%.

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF WHEAT SEED TREATED WITH TALC DUSTS CONTAINING PHYTOHORMONE, POTASSIUM NITRATE, AND AN ORGANIC MERCURIAL DISINFECTANT

Source of variance	Degrees of freedom	Mean square Experiment 1 (Indolylacetic acid)					Mean square Experiment 2 (Potassium naphthylacetate)				
		Germin- ation rate	Final germin- ation count	Air-dry weight of:			Germin- ation rate	Final germin- ation count	Air-dry weight of:		
				Whole plants	Stems	Roots			Whole plants	Stems	Roots
Replicates	9	0.1786***	31.93**	1.02**	0.680**	1.02**	0.0504***	8.84	0.624***	0.3097**	0.0586**
Treatments:											
Untreated versus treated	1	.0001	0.00	0.05	.003	0.05	.0164*	0.10	.047	.0661	.0018
Phytohormone	4	.0025	2.28	.54	.160	.16	.0011	19.39*	.025	.0077	.0046
Potassium nitrate	1	.0005	0.50	.76	.043	.30	.0003	13.52	.128	.0633**	.0123
Ethyl mercuric bromide	1	.0280***	118.58**	1.11	.249	.10	.0523***	21.78	.054	.0003	.0005
Interactions:											
Phytohormone X potassium nitrate	4	.0014	7.16	.66	.212	.26	.0017	11.83	.035	.0127	.0070
Phytohormone X ethyl mercuric bromide	4	.0024	5.34	.34	.062	.14	.0020	3.19	.050	.0193	.0018
Potassium nitrate X ethyl mercuric bromide	1	.0014	0.02	.51	.089	.13	.0104	27.38*	.197*	.1220**	.0059
Phytohormone X potassium nitrate X ethyl mercuric bromide	4	.0010	.43	.47	.178	.13	.0053	2.42	.014	.0134	.0022
Error	180	.0019	3.56	.32	.112	.12	.0030	6.42	.045	.0088	.0078

* Exceeds mean square error, 5% level of significance.

** Exceeds mean square error, 1% level of significance.

*** Exceeds mean square error, 0.1% level of significance.

The average effect of organic mercury treatment was a reduction in germination rate per cent from 78 to 75.5. The average final germination was approximately 91%. Neither the mercurial nor potassium nitrate, applied separately, changed this, but, when these chemicals were used in combination, germination fell to 88%.

Neither organic mercury nor potassium nitrate treatment, alone, affected the air-dry weight of plants from 50 seeds, which averaged 2.70 gm. In combination these chemicals reduced the average weight to 2.61 gm. A similar interaction was demonstrated by the air-dry weight of stems from 50 seeds which averaged 1.90 gm. in the absence of both organic mercury and potassium nitrate. Combination of organic mercury and potassium nitrate resulted in a weight of 1.84 gm.

Dust treatments, on the average, reduced germination rate from 81%, for the untreated control, to 77%. Conversely, the air-dry weight of stems from 50 seeds was 1.80 gm. for the untreated, but averaged 1.89 gm. for the dust treated.

Phytohormone treatments, alone, or in combination with nutrient and disinfectant chemicals, failed to have any marked effect on germination or early growth of wheat under the conditions of these experiments. It seems probable that instances of stimulation from treatment of cereal seeds relate to the circumstances under which the observations were made.

It is of interest to note that the organic mercurial disinfectant tended to retard the rate of germination but increased the final germination count. Such increases have been noted in the literature (5). Whereas potassium nitrate treatment failed to show the stimulatory effect of the type noted in earlier experiments (12), it did interact unfavourably when used in conjunction with the organic mercurial. In contrast, similar combinations applied to plant stem cuttings have given favourable responses (14).

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THE INHERITANCE OF FRUIT SIZE IN THE TOMATO¹

BY L. BUTLER²

Abstract

It is pointed out that size data from over 50 tomato crosses are explained by the assumption of the geometric action of size factors but not by a simple additive theory.

The fact that the F_1 results fitted such a theory was pointed out in a previous paper when the theory was proposed. The analysis is here extended to the F_2 generation and to cell size measurements.

The use of the geometric scale introduces regularity into the otherwise unpredictable F_2 segregations, and they become amenable to a simple logarithmic scheme. Analysis by such a scheme indicates that differences in cell number or ovary size are caused by the segregation of three to five pairs of major genes, whereas mature cell size differences seem to be brought about by the segregation of at least twice as many factors.

Final weight is thus the resultant of the proportionate action of the following factors:—

1. The number of mitotic divisions in the pre-anthesis period and therefore the number of cells at anthesis.
2. The cell expansion after anthesis.
3. Fruit shape, locule number, and other size-modifying effects.

The science of genetics has been very severely criticized because it deals only with superficial characters and with freaks that are not essential for the continuance of the species; therefore it is gratifying to see the revival of interest in the inheritance of quantitative characters. Size is a highly complex and important character from the practical standpoint as well as from the pure science viewpoint. As it is the chief concern in the problem of hybrid vigour any rational rules that can be formed about its control and its determination will be of great assistance in solving this problem. Size is, moreover, one of the better known examples of multiple factor inheritance; since there is very little reliable information on this subject, the data presented here may help to solve some of the many problems connected with such a type of inheritance.

The tomato fruit, because of its wide range in size and the presence of marker genes in most of its chromosomes, presents excellent material for the study of size inheritance. The inheritance of fruit weight has been studied by Groth (10, 11, 12), who found that the F_1 weight was the geometric mean of the two parental weights. His proposed hypothesis of the "Golden Mean" in size inheritance was poorly received. Lindstrom (15), also working with fruit weight, found that the F_1 mean was much closer to the smaller parental value; he postulated the dominance of small size. The histological basis of differences in fruit size was investigated by Houghtaling (13) and Butler (2),

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both of whom arrived at the same conclusion by different methods of approach. They stated that prior to anthesis, since cell volume at that time was the same for all varieties, increase in size was by cell division so that ovary size is directly related to cell number. After anthesis growth is due chiefly to cell enlargement.

Recently MacArthur and Butler (17) as the result of extensive investigations of the inheritance of fruit size in the tomato have put forward a theory of inheritance based on geometric growth processes. By four different approaches they showed that inheritance of fruit size is of a proportionate or geometric nature and not the simple additive type which is usually postulated.

When parents with fruit of two different sizes were crossed the fruit size in the F_1 was close to the geometric mean of the two parental sizes. This was explained by the fact that the cell number of the fruits of parents and F_1 are in geometric progression, so that the number of cells in the F_1 is caused by an intermediate number of cell divisions. It was further intimated that inheritance of cell size seems to be of a somewhat similar type, but the data on cell size were not extensive. During these experiments further data were collected and found to substantiate these findings.

In this paper the analysis will be carried into the F_2 .

The Segregation of Weight in the F_2

In their paper (p. 257), MacArthur and Butler give curves showing the F_2 frequency distribution of the fruit weights of two crosses in each of which approximately 1000 F_2 plants were grown. Examination of these curves shows that they are both strongly skew, g_1 being +1.105 and +1.596. Such a result is typical for the weight segregation in 56 crosses examined in this study. When the logarithms of the weights are plotted instead of the weights themselves, the curve becomes normal.

The regularity introduced into the measurements by using logarithms can also be illustrated in the following manner. Taking the two parental weights, the two extremes of F_2 range, and the F_2 mean the following five values are obtained:

P_1		F_2 Segregants			P_1
Small		Smallest	Mean	Largest	Large
1.1		2.3	7.6	25	56
	1.2	5.3	17.4	31	

The second line is obtained by subtracting consecutive terms of the first row. The two inner values represent the spread of the range on each side of the mean. In a symmetrical distribution these two values would be the same, so that the departure from equality is an indicant of skewness. The two outer values show the distribution of the segregation with respect to the two parent values. If the inheritance was of the typical blending type these two values would be equal, but it is easy to see that the values are very unequal. The smallest F_2 fruit is almost 30 times closer in size to that of the smaller parent than the largest F_2 fruit is to that of the larger parent.

If the logarithms of these values are taken, the following series is obtained:

P_1	F_2 Segregants				P_1
Small	Smallest	Mean	Largest	Large	
0.041	0.362	0.881	1.398	1.748	
	0.321	0.519	0.517	0.350	

It will be seen that the logarithms of the numbers fit into a definite series. The F_2 segregation occupies the central part of the parental range, the mean being 0.840 from the small parental weight and 0.867 from the large parental weight. The F_2 mean is now in the centre of the distribution instead of being closer to the smallest segregant.

The use of logarithms on the data for other crosses and the backcrosses produces the same type of regularity. Such behaviour leads to the assumption that the growth processes controlling fruit size act in a geometric manner, and with this in view, the segregation of these processes has been examined.

The Inheritance of Ovary Size in the F_2

It has been previously shown (2), because cell volume is the same in all varieties at anthesis, that ovary size is proportional to cell number. Therefore, because of its convenience, ovary size may be used as an indicator of cell number segregation in the F_2 . The distribution of ovary sizes at anthesis, like that of fruit weights, is strongly skew in a positive direction but becomes normal when plotted on logarithmic paper. There is, however, one important difference which is readily apparent from an examination of Table I. In

TABLE I
SEGREGATION OF OVARY DIAMETERS

	Red Cur- rant			Yellow Cherry Burbank Preserv- ing	Yellow Peach		Pink Peach	902 selec- tion	Devon Sur- prise		Tan- gerine
Ovary diameter	1 0	1 1	1 2	1 3	1 4	1 5	1 6	1 7	1 8	1 9	2 0
463 R.C. × Pk. Pch.	13	45	50	35	14	7					
3313 R.C. × 902 sel.	4	9	23	18	8	5	1	1			
3210 R.C. × Tang.	4	29	73	57	26	9	2				
3218 B.P. × 902 sel.			2	3	20	11	18	10	3		
3728 Y.C. × Tang.				1	15	40	33	14	6	3	2
522 Y.Pch. × Devon S					9	51	59	23	4		

most of the crosses the parental values are recovered, indicating that ovary size is controlled by fewer factors than fruit weight. The parental fruit weights were not recovered even when 10,000 plants of the F_2 generation were grown. A second feature of these distributions is the strong positive skewness indicating either geometric action of the genes concerned or the dominance of small size. In an attempt to assess the probable number of

factors operating in these crosses, use is made of the binomial distribution. It is known that non-dominant genes of equal effect and equal frequency are distributed in classes according to the expansion of the binomial $(a + b)^n$. The number of genetic classes of different numerical value is, accordingly, $n + 1$ and the frequency of each class is given by the numerical value of the coefficient of each term in the expanded binomial. The number of pairs of allelomorphous genes is $n/2$ and the least number of individuals required to include all classes is 2^n .

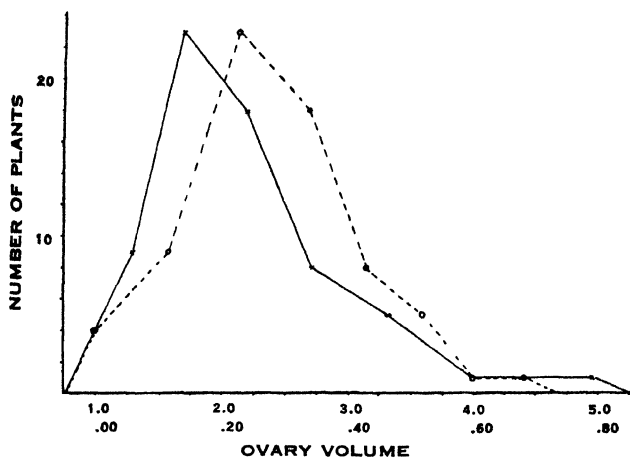


FIG. 1. Distribution of ovary volumes in the F_2 of cross 3313 (Red Currant \times 902 selection). — x — Arithmetic scale. -- o -- Logarithmic scale.

Advantage may be taken of this last statement to estimate the number of genes controlling the difference in parental ovary sizes in cross 3313. Assuming that the large parent carries all the large-size producing genes and the small parent the less effective allelomorphs of these, then the extremes of range possible in this cross are the two parental classes. Then, since all classes were observed when only 69 individuals were measured, it is concluded that about three pairs of genes ($2^6 = 64$) are concerned here. Next the abscissa values or ovary sizes that correspond with the theoretical gene frequencies must be obtained. Now since gene action as far as ovary volume is concerned is geometric, a geometric or logarithmic scale must be used. Assuming as before that the two parental values are the upper and lower limits for this segregation, then the range of the logarithmic scale is $0.612 - 1.982 = 0.630$. To test the hypothesis of three pairs of genes, seven ($n + 1$) equally spaced abscissal values are obtained by dividing the range by n and getting 0.105 as the interval between classes. Then starting from the value of the smaller parent and adding 0.105 each time the required class values given below are obtained.

Class value	1.982	0.087	0.192	0.297	0.402	0.507	0.612
Observed frequency	1	5	12	23	15	9	3
Theoretical	1	6	16	21	16	6	1

Similar schemes may be worked out for the rest of the crosses. With the exception of those involving Tangerine, the theoretical and observed values in all crosses are similar. When Tangerine is one of the parents the distribution is biased towards the smaller parent. This bias may be explained by assuming multiple-allelomorphic action of some of the size genes.

The Inheritance of Mature Cell Size in the F_2

The size of cells of the locule wall is an inherent character of the variety, and the F_1 data indicated that a geometric type of gene action was responsible for its inheritance. From this it is deduced that the segregation in the F_2 will skew when plotted on an arithmetic scale, but will become normal when plotted logarithmically. Plotting the data for crosses 3313 and 3210 in such a manner demonstrates the correctness of the preceding deduction. The logarithmic curves for these two varieties are given in Fig. 2, and it will be observed that both are very similar to the normal curve. The only abnormality noticed is the erratic nature of segregation at the upper end of the range.

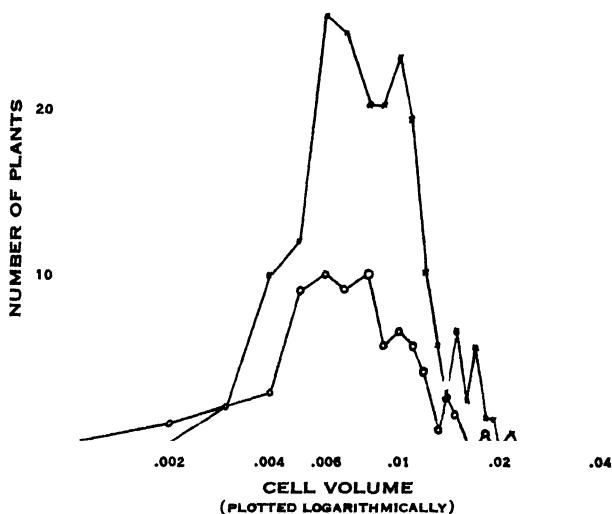


FIG. 2 Segregation of cell volume in the F_2 . —○— 3313 F_2 ($R C \times 902$ selection). —x— 3210 F_2 ($R.C \times Tangerine$).

As the parental cell sizes are not recovered in either of the crosses, the method adopted for predicting the number of segregating genes for ovary volume, cannot be used here. Other methods have been tried but none have proved satisfactory. Most of the known methods are based on the surmise that size genes have an equal effect. Such an assumption does not seem valid for cell expansion.

Many of the methods of estimating the number of genes are based on the standard deviation. There are two methods of obtaining this statistic; it may

be obtained from either the arithmetic measurements or from their logarithms. It has been the custom to use the arithmetic series for such calculations, but the asymmetry of the arithmetic curve in tomato leaves such a procedure open to grave objections. On the other hand the use of the logarithmic series for the calculation of the standard deviation is also objectionable because of the arithmetic nature of environmental variation. It is always assumed that environmental variation is arithmetic, and such an assumption is indicated in tomato by the normality of the arithmetic curves for the P_1 and F_1 generations; Kapteyn (14), however, has pointed out that the geometric is more plausible. The normality of the curves may be the result of lack of numbers, large populations being required to distinguish between the two in such cases. Since neither method is without objection an example using each method will be given and the results by the two methods will be compared.

In cross 3313 the standard deviation calculated from the arithmetic series is 0.00416, and the mean of the series is 0.00876 cu. mm. The two parental values are 0.001 cu. mm. and 0.059 cu. mm. From a table of probability integrals, the chances of obtaining the parental values and the number of plants that must be grown in order to obtain them can be worked out. The cell size of Red Currant, the smaller parent, is 2.1 standard deviations from the mean, and from a table of probability integrals it is found that cells of this size and smaller would be expected to occur twice in each hundred plants in the F_2 . Turning next to the upper end of the range and to the larger parent, it is found that the value for cell size of the large parent is 12 standard deviations above the mean of the F_2 . Although with the normal curve infinitely large values can occur, the chances of getting a value even six standard deviations away is 5,000,000,000 : 1, and the chances of getting a deviation equal to 12 standard deviations is so infinitely small that the value is not included in the ordinary tables.

The standard deviation of the logarithmic series of cross 3313 is 0.226 and the mean is 0.897. The two parental values are 0.000 and 1.771. The chances of obtaining the small parental size are 4 in 10,000 and the chances of obtaining the large parental size are 7 in 10,000. The chances of getting either parental size are both equal. The probability of recovering either of the parental sizes in the F_2 is quite small in samples of the size usually grown, but if an F_2 generation of 1000 plants was grown there would be an even chance of getting one plant with the cell size of one or other of the parents. These considerations lead us to prefer the standard deviation of the logarithms when working this statistic for use in estimating the number of factors involved in size crosses. In his selection experiment with mice, Goodale (9) based all his calculations on the arithmetic scale and concluded that his selected mice must have mutated in order to reach their final size. If his figures were recalculated on a geometric scale it would not be necessary to postulate any new mutations.

Discussion

Consideration of the tomato data and a critical examination of the literature causes the author to raise several objections to the simple additive scheme of inheritance. These objections are:

1. The inapplicability of the arithmetic mean to measurements of all characters of the individual. If linear measurements are studied and found to fit the arithmetic mean, then it is impossible for weight, volume, or area to fit the same arithmetic scheme since they depend upon a power of the linear measurements. An arithmetic scheme raised to any power other than one is no longer an arithmetic scheme; the interval between consecutive members of the series is now different. This fact has not been recognized by other investigators although it was pointed out by Kapteyn, in reference to the normal curve, as early as 1916. The use of the geometric mean is free from this objection as *a geometric series can be raised to any power and still remain a geometric series* with equal logarithmic intervals between successive terms.

2. The F_1 is closer to the geometric mean than it is to the arithmetic mean of the parental values. Groth (11, 12), and Dale (5), both stated that their data were fitted much better by the geometric mean than they were by the arithmetic one. Sinnott (24) found that the percentage method fitted his cucurbit data, whereas a simple additive scheme did not. MacArthur and Butler (17) gave extensive data for fruit weight in the tomato to show that the F_1 mean is the geometric mean of the parent means, and not the arithmetic mean of the parental averages. They also presented a working hypothesis of size inheritance in which they claimed the geometric mean was a more rational value than the arithmetic average. If the data in the literature are examined in the light of this hypothesis, it is found that, although not recognized by the investigator, the data fit the geometric mean, as well as, or even better than they do the arithmetic mean.

There are two main reasons why investigators have failed to notice the similarity between their results and the geometric mean. In the first place, when the differences in size between the two parents are not great, there is very little difference between the arithmetic and geometric mean; either could be applied equally well to the data. In the second place there are a number of results in which dominance of small size has been postulated; such results are usually compatible with a geometric interpretation.

As examples of the first case may be quoted the work of Sosa-Bourdouil (26) with plants, and Wexelsen (27) with animals. Sosa-Bourdouil, working with broad beans, postulated an intermediate value for the F_1 , but the F_1 weight that he obtained, i.e., 0.8 gm. from a cross in which the parental weights were 1.4 and 0.49 gm., is closer to the geometric mean of 0.83 than it is to the arithmetic mean of 0.95 gm. In pigeons, Wexelsen found the F_1 beak length in a cross of Field (23.8 mm.) \times Bagadette (34.0 mm.) to be 28.4 mm. The geometric mean was 28.4 and the arithmetic mean, 28.9 mm. It will be seen that although the geometric mean fitted the data better



than the arithmetic mean, there was really no significant difference between the two means. In examples of this type the arithmetic mean can be expected to fit the data just as often as the geometric does; therefore they have no diagnostic value.

As an example of dominance of small size Maw's (18) work on domestic fowl can be quoted. He said, "There is a tendency for the F_1 mean weight to approach the mean weight of the smaller parent". Examination of his figures show that they fit in well with the geometric mean. In his cross of Brahma (3350 gm.) \times Sebright (708 gm.), the F_1 weight was 1612 gm. The geometric mean is 1590, and the arithmetic mean is 2129 gm. The geometric mean gives a good fit without postulating dominance, but the arithmetic mean does not.

3. The F_2 frequency curves for weight, cell size, and cell number, were all strongly skew in a positive direction when plotted in arithmetic metre, but became normal when plotted on a logarithmic scale. Such a finding has two interpretations: either gene action is proportionate and not additive or small fruit size is dominant over large fruit size. The second interpretation is not to be favoured as it assumes the dominance to be only partial and of exactly such a degree that the F_1 mean is always close to the geometric mean of the two parents. The chances of such an occurrence are remote, especially when one considers that genes controlling several processes would have to show the same type of dominance behaviour. Sinnott (24) obtained the same type of curves and postulated proportionate action of genes to explain the asymmetry.

The asymmetry of the F_2 offers a grave objection to the theory of simple additive action of size genes. The fact that the use of logarithms makes the skew curve symmetrical is one point in favour of the geometric or proportionate action of size genes.

4. The unreasonable nature of probability integrals calculated from the standard deviations of the additive scheme. The standard deviations for cell size show that there are 10,000,000,000 more chances of obtaining the small parental size than there are of obtaining the large parental size. The use of standard deviations from the logarithmic series shows these chances to be equal. The logarithmic or proportionate interpretation, therefore, appears to give more rational results.

5. The proportionate nature of physiological effects. Qualitative genes that affect fruit size were found by MacArthur and Butler to produce a constant percentage increase or decrease (17). This is compatible with the work of Robb (22), who found that the percentage growth rate is identical for Flemish giant, hybrid, and Polish rabbits from birth to the onset of puberty.

The interpretation placed on the results obtained in tomato is that the action of qualitative genes is to produce an increase or decrease proportionate to the size attained in the absence of these genes. The question therefore is raised, "If qualitative genes affect fruit size in this proportionate manner, why should typical size genes as such not do the same?"

The proportionate nature of the increase is also to be seen in linkage studies where the linkage of a quantitative trait with a qualitative character is investigated. The work of Sax (23), Sirks (25), and Rasmusson (21) shows the proportionate nature of the linkage effect, although the investigators themselves give only the absolute increase.

The geometric or proportionate nature of the size effects of known genes makes it extremely probable that size genes as such also show this proportionate effect.

These five objections make it clear that the tomato data, as well as most of the size inheritance data in the literature, are not compatible with the simple additive theory of gene action. The geometric hypothesis is open to none of these objections and it can now, it is thought, be adopted in place of the additive one.

Recently Powers (19, 20) in a study of the interactions of locule number, fruit shape, and fruit size came to the conclusion that "The effects of the genes differentiating number of locules were geometrically cumulative. Also this is true of the effects of genes differentiating fruit size". His quantitative results are in agreement with those of MacArthur and Butler and are additional evidence in favour of the geometric hypothesis of size inheritance. /

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THE REACTION OF *FRAGARIA VIRGINIANA* TO THE VIRUS OF YELLOW-EDGE¹

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Abstract

When runner-grafted to domestic plants of the strawberry varieties Premier and Glen Mary (symptomless carriers of the virus of yellow-edge) clones of the common wild strawberry, *Fragaria virginiana* Duchesne, were found to vary widely in their resistance and susceptibility to the disease. Certain clones of noticeably more delicate growth type, proved to be very highly susceptible to deterioration and exhibited complete symptom-expressing propensities. Other clones, characterized by a particularly robust type of vegetative growth, although readily susceptible, showed capacity for at least partial recovery, tending to react more like the English indicator variety, Royal Sovereign. Still other clones, also of the robust type, showed resistance that was virtually complete; thus they were eliminated from plants of the "carrier" class.

Introduction

Before continuing the strawberry virus investigations carried out at St. Catharines from 1933 until 1935 (4), it became necessary to find an indicator plant to replace the English variety, Royal Sovereign, which had been used in the earlier experiments. Under Ontario conditions, plants of this variety proved so susceptible to attack by certain insect pests and fungous diseases that it was found impossible to maintain them for any considerable time at the level of health and vigour required of plants to be used in virus research. This necessitated frequent renewals of stock by importation from England and almost invariably only a few, if any, plants of each shipment survived the long period in transit. Since Harris (3) in England had demonstrated that species of *Fragaria*, namely, *F. vesca* and *F. virginiana* could be used as indicator plants, it was decided to investigate the possibility of using the same two species, both of which are indigenous to Ontario, in further work at St. Catharines. The results which have been obtained from investigations carried out during the past three years are reported in the present paper.

Experiments—1937

In May, 1937, 50 plants of *F. virginiana* and the same number of *F. vesca* were transferred to the greenhouse from their natural habitat. At the same time, Premier plants chosen from stocks obtained from two widely separated commercial sources in Ontario (hereinafter designated Premier A and Premier

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B), as well as plants of the variety Glen Mary, were also grown in the greenhouse. By early July of 1937, both wild and domestic plants were producing runners in profusion but those of *F. vesca* were so fine and thread-like that they could not be used for grafting and the species was discarded.

Using the method of runner inarching described by Harris in 1932 (1) and by Harris and Hildebrand in 1937 (4), runners of six, ten, and nine different

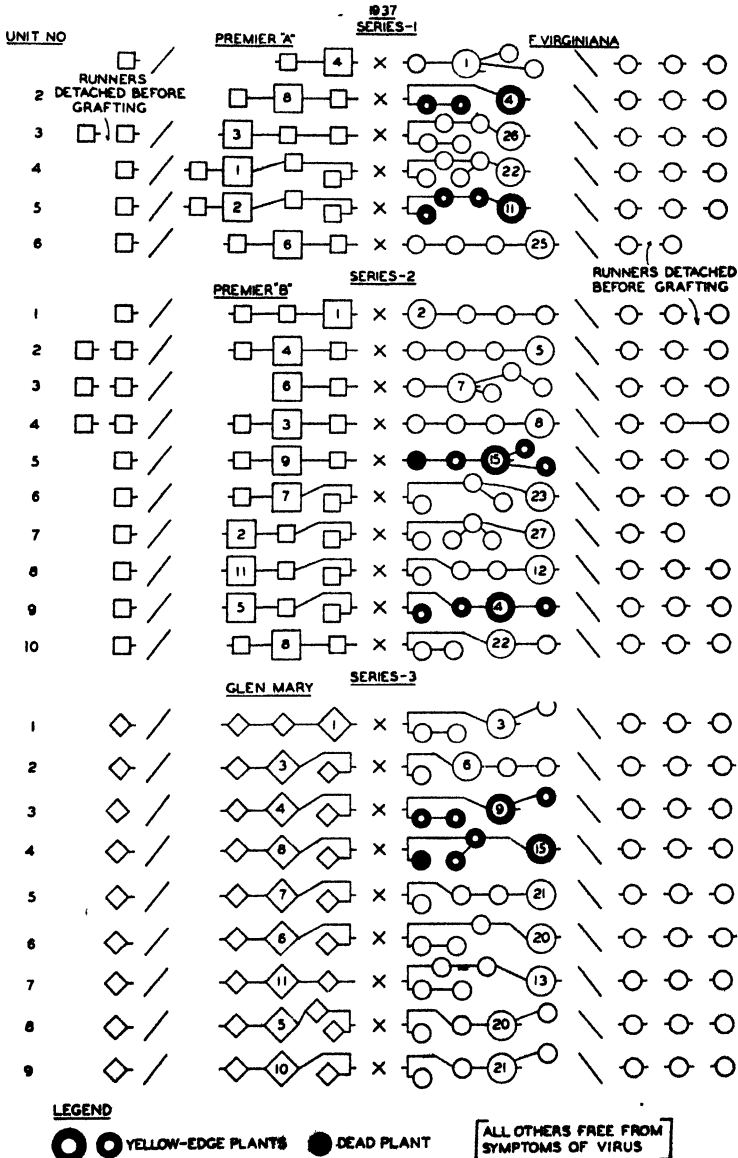


FIG. 1. Various types of graft combinations or graft units together with results obtained in 1937 grafting experiments involving different clones of *F. virginiana* and domestic plants of unknown virus potentiality.

clones of Premiers *A* and *B* and of Glen Mary, respectively, were grafted to those of *F. virginiana* plants representing 20 different clones. In some, the stolons grafted were those produced by parent plants (Fig. 1, Series 2, Unit 1). Frequently, stolons of *F. virginiana* grew to a length of several feet with "runner buds" occurring at intervals. From these it was possible to strike a number of runners which constituted a chain of plants connected in series by a common stolon. The continuation of the latter from the terminal plant of the series was used for grafting (Fig. 1, Series 1, Unit 6). In a graft unit of the latter type it was thought that in the event of transmission of virus from the Premier or Glen Mary component it might be possible to trace the rate of spread of the disease by noting the time interval elapsing between symptom expression in successive "links" in the chain of *F. virginiana* plants organically united in series.

At the commencement of the experiment all plants involved were in an apparently healthy condition. To avoid complications that might arise from a root rot factor, all runner progeny were grown in autoclaved soil. The 25 graft units completed in July, 1937, were kept under constant observation in the greenhouse until June, 1938. The arrangement and number of plants involved in the 1937 series, together with the end results of the experiment are shown graphically in Fig. 1.

RESULTS

The *F. virginiana* components of six graft units developed symptoms which were indistinguishable from those of yellow-edge on Royal Sovereign. Furthermore, the virus was transmitted to only four of the 20 different clones of *F. virginiana*, namely, 4, 9, 11, and 15 (Fig. 1). Of the four apparently susceptible clones, 4 and 11 exhibited a particularly robust vegetative growth and without close examination could scarcely be distinguished from plants of cultivated varieties. Clones 9 and 15, on the other hand, were characterized by a noticeably more fragile type of growth, their leaves, petioles, and stolons being much less robust than those of the other clones—including 4 and 11. These differences gave evidence of the wide variations that must exist within the species as far as vegetative characters are concerned.

The reaction of plants of the wild species to yellow-edge seemed correlated in part, at least, with vigour of vegetative growth. For example, the disease was much more severe in plants of Clones 9 and 15 than in those of 4 and 11. Moreover, plants of Clones 9 and 15, once infected, remained permanently in the dwarfed, "flattened" condition, whereas those of Clones 4 and 11 showed capacity for at least partial recovery from the disease, especially during the period of renewed growth activity in the spring of 1938. Thus, plants of Clones 4 and 11 tended to react more like those of the English indicator variety, Royal Sovereign.

In Series 3, Graft unit 4 (Glen Mary 8 \times *F. virginiana* 15), yellow-edge symptoms became apparent on the *F. virginiana* plant closest to the graft union about 22 days after the date of grafting. In the corresponding *F. vir-*

giniana plant, Series 2, Graft unit 5 (Premier B 9 \times *F. virginiana* 15), the symptoms became distinguishable about 30 days after the graft had been made. In no other case did symptoms show up in *F. virginiana* plants grafted directly to either Premier or Glen Mary plants in less than 60 days after grafting. This circumstance and the fact that before the expiration of the experiment two plants of Clone 15 died (Fig. 1, Series 2, Graft unit 5; Series 3, Graft unit 4), made it appear that this clone was even more susceptible than Clone 9 and much more so than Clones 4 and 11.

All progeny of the apparently susceptible Clones 4, 9, 11, and 15 that had been detached previous to grafting, remained free from symptoms of yellow-edge, as did all other plants, wild and domestic, involved in the experiment. Non-appearance of virus symptoms could not be attributed in a single instance to faulty technique since in the 25 graft units organic union had taken place at the point of grafting.

DISCUSSION

Since previous work at St. Catharines (4) had demonstrated that Premier and Glen Mary plants both possess the symptomless-carrier capacity for yellow-edge and since Harris (3) had shown also that *F. virginiana* "proved to be highly susceptible to deterioration and exhibited all symptoms" (of yellow-edge), no especial interest attached to the fact of merely having confirmed these findings. It did seem of particular interest, however, that as the results suggested, within the species there might exist clones or strains apparently differing so widely in their resistance and susceptibility to the disease. It was realized, of course, that non-appearance of yellow-edge symptoms in the *F. virginiana* components of 19 of the 25 graft units might have been owing to the fact that certain of the domestic plants were free from infection, as had been found in previous work; nor was it impossible that certain of the wild plants might have possessed the symptomless-carrier capacity to as high degree as the Premier and Glen Mary plants to which they were grafted; in that case, though infected, they would not show symptoms. To try to arrive at the true explanation of the observed phenomena, further work was carried out in 1938.

Experiments—1938

Early in July, 1938, there was completed a fourth series of grafts (Fig. 2) in which progeny of plants of the commercial varieties, Glen Mary and Premier, that in 1937 *had passed on* the virus, were grafted to *F. virginiana* plants of the clones that had *failed to take* the disease. Reciprocally, Glen Mary and Premier plants that apparently *had not passed on* the virus were grafted to healthy *F. virginiana* plants of the clones that *had taken* it from other plants of these two varieties. Altogether, as reference to Fig. 2 will show, Series 4 comprised 12 such graft units.

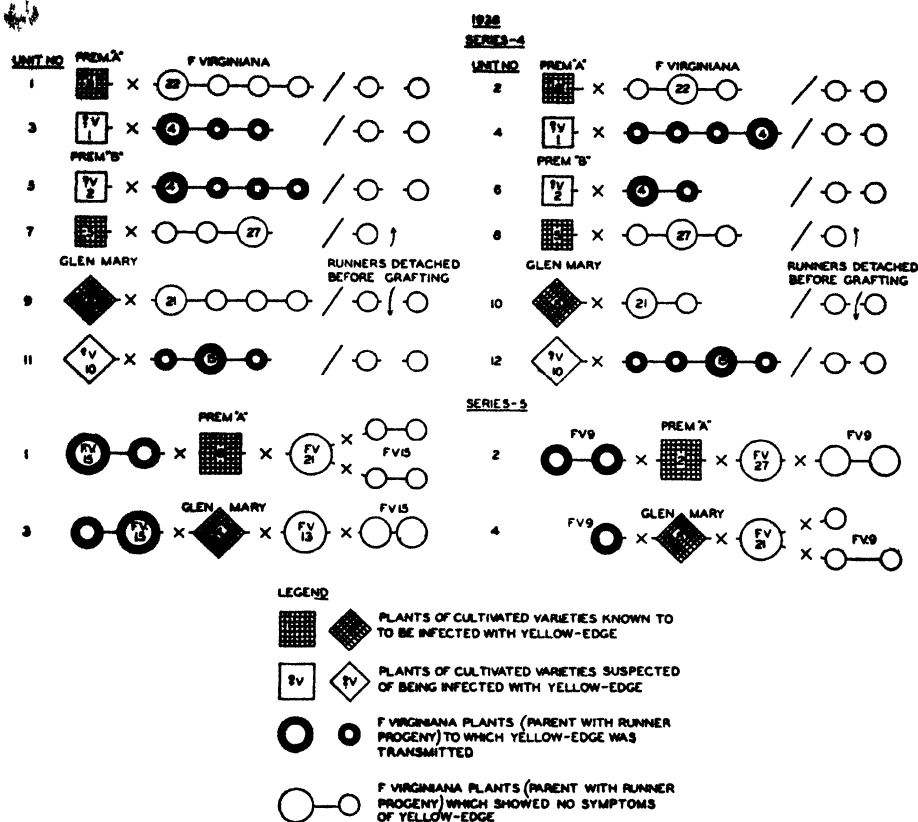


FIG. 2. Various types of graft combinations or graft units, together with results obtained in 1938 grafting experiments involving presumably resistant and susceptible clones of *F. virginiana* and domestic plants of known and suspected virus potentiality.

Although the number of domestic plants of known history, with runners suitable for grafting, was limited, sufficient were still available to complete the four additional graft units which comprised Series 5. This series was designed primarily to find out whether certain clones of the wild plants which up to that time had failed to show symptoms of the disease, were really resistant to it, or like certain clones of the domestic varieties, were susceptible but possessed the symptomless-carrier capacity. Details of the arrangement of plants and scheme of grafting are shown in Fig. 2, Series 5, Graft units 1 to 4. A domestic plant, proved in 1937 to be infected with yellow-edge, was grafted by one runner to a wild plant known to be susceptible and by another to a presumably resistant wild plant. The "resistant" wild plant in turn was grafted to one or (if possible) two of the susceptible wild plants. Thus was constituted a "chain" of plants the respective "links" being as follows: susceptible wild plant, domestic plant of known virus content, resistant wild plant, and again the susceptible wild plant. The results obtained in the 1938 grafting are shown graphically in Fig. 2.

RESULTS

Series 4

F. virginiana plants of Clones 21, 22, and 27, when grafted to Premier and Glen Mary plants proved by the work of 1937 to be infected with yellow-edge, failed to show any symptoms of the disease. It remained to be seen whether or not these *F. virginiana* clones had become infected. If so, it could then be assumed that they must possess the symptomless-carrier capacity to as high degree as the domestic plants to which they were grafted. If not, they must be completely resistant. In each case where *F. virginiana* plants of presumably susceptible Clones 4 and 15, were grafted to Premier and Glen Mary plants of as yet unknown virus potentiality, they developed characteristic symptoms of the disease, thus confirming earlier indications that they were really susceptible to infection by the virus and by the same token proving that Premier A 1, Premier B 2 and Glen Mary 10 were infected with the disease. As indicated in Fig. 2, no symptoms of yellow-edge were noted on runner plants of any of the *F. virginiana* clones, detached before the grafts were made. The above results seemed to furnish clear evidence that clones of the wild plants differed markedly in resistance and susceptibility to the disease.

Series 5

In all four cases *F. virginiana* plants of susceptible Clones 15 and 9, grafted directly to the virus infected domestic plants, developed characteristic symptoms of the disease. In contrast, however, *F. virginiana* plants of the "resistant" Clones 13, 21, and 27, also grafted directly to the virus infected domestic plants, showed no evidence of the disease throughout the period of observation which lasted from the time the grafts were made in July, 1938, until June of the following year. That plants of Clones 13, 21, and 27 must be regarded as possessing resistance that is virtually complete, is proved by the fact that plants of the highly susceptible Clones 9 and 15, which formed the final link in the chain of grafted plants, showed no evidence of the disease. Examination showed that organic union had taken place at each point of grafting so that failure of transmission could not be attributed in a single instance to faulty technique. The same was true for all graft units of Series 4.

Experiments—1939

As in previous years progeny of the various clones of wild and domestic plants were carried through the winter of 1938–39. In the spring and early summer of 1939, the wild plants produced an abundance of runners suitable for grafting. In marked contrast, however, the domestic plants produced so few runners that it was impossible to build up a population of these plants and further work could not be carried out as planned. If additional grafting were to be done, the only alternative was to resort again to domestic plants of unknown virus potentiality. Late in June, Glen Mary and Premier plants, the latter, as in 1937, obtained originally from two widely separated sources

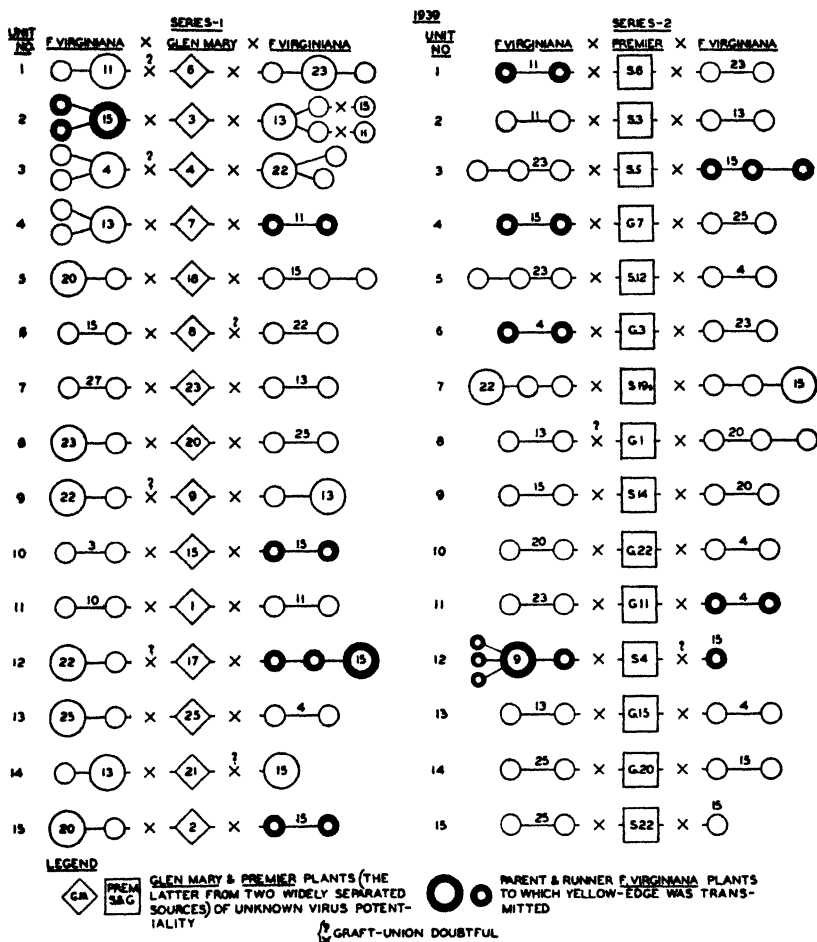


FIG. 3. Various types of graft combinations or graft units, together with results obtained in 1939 grafting experiments involving presumably resistant and susceptible clones of *F. virginiana* and domestic plants of unknown virus potentiality.

in Ontario, were brought into the greenhouse from outdoor plots. As runners from these plants became available, two series, each comprising 15 graft units, were completed. In Series 1, a runner from each of two different clones of *F. virginiana* was grafted to each of a pair of runners produced by a Glen Mary plant. Series 2 was the same except that Premier plants were used, the designations S and G indicating their different sources. As reference to Fig. 3 will show, both of the *F. virginiana* components in certain of the graft units (Series 1, Unit 8) represent resistant clones. In other graft units (Series 2, Unit 12) both of the *F. virginiana* components represent susceptible clones. In still others (Series 1, Unit 15), one *F. virginiana* component represents a resistant clone, the other a susceptible one. It will also be noted that in a total of 60 grafts, the success of the graft in eight was doubtful. These eight possible failures are probably owing to the fact that because of the change in

plans necessitated by the non-production of runners by the domestic plants used in 1937 and 1938, a considerable period elapsed between the time of runner production by the wild plants and the formation of runners by the new series of domestic plants. This meant that in a number of grafts the runners used, particularly those of the wild plants, had passed the stage of succulence most conducive to success in grafting. Furthermore, some of the grafts could not be completed until about the middle of August. Previous experience has shown that much greater success is obtained when grafts are made earlier in the growing season. The results obtained in the 1939 experiments are shown graphically in Fig. 3.

As reference to Fig. 3 will show, in the 43 grafts in which representatives of presumably resistant clones were grafted to the domestic plants of the two varieties, symptoms of yellow-edge did not appear on any of the wild-plant components, thus confirming previous evidence as to their resistance to the disease. In 12 of the 27 grafts of representatives of susceptible clones to the domestic plants, characteristic symptoms of yellow-edge resulted. In three of the remaining 15, involving presumably susceptible clones (Series 1, Units 1, 3, and 14), failure of transmission might reasonably be attributed to failure of organic union at the point of grafting. In the non-appearance of yellow-edge in the remaining 12 grafts of *F. virginiana* plants from susceptible clones and Premier and Glen Mary plants, it is probable that the latter were not infected with the virus. In previous work (4) it was found that certain Glen Mary and Premier plants apparently were not infected since they did not transmit the disease to plants of the highly susceptible variety, Royal Sovereign.

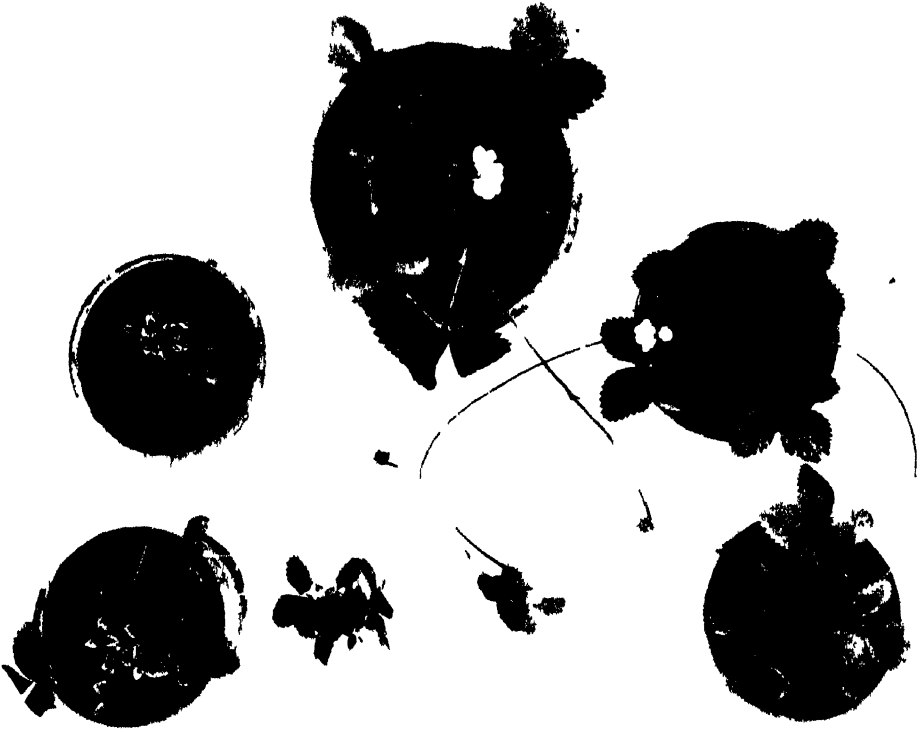
Attention should be directed to Unit 2 of Series 1. Glen Mary 3 has transmitted the virus to susceptible *F. virginiana* 15. However, *F. virginiana* 13 was evidently completely resistant to the disease, otherwise the virus would have been transmitted through it to *F. virginiana* 11 and 15, both of which are susceptible. This unit offers further confirmation to the conclusion drawn from the results of Series 5, 1938, that certain strains of *F. virginiana* are virtually completely resistant to the disease.

As typical of a graft unit showing very clearly two clones of the wild plant differing markedly in their resistance and susceptibility to the disease, Unit 6, Series 2 (Fig. 3) was photographed (Plate I) in October, 1939, approximately three months after the grafts had been made.

Discussion

In England where virus diseases are "a serious and widespread contributory cause of varietal deterioration" (3, p. 201) intensive research has been carried out almost continuously since 1932 when Harris (2) identified yellow-edge on the variety Royal Sovereign. The study of yellow-edge, at first centring about the above named variety, has been extended to include other commercial varieties and species of *Fragaria*. The results as summarized recently by

PLATE I



Graft unit 6, Series 2, 1939, typical of many others showing resistant (right) and susceptible (left) clones of *F. virginiana* grafted to yellow-edge infected Premier plant (upper centre) of the "carrier" type. Unit photographed approximately three months after grafts were made.

Harris and King (5) are in part as follows— "*Fragaria chiloensis* reacted as a symptomless carrier of the disease with high resistance to deterioration and at the other end of the scale *F. virginiana* (cultivated in this country as "Little Scarlet") and the native woodland species, *F. vesca*, were found to combine a high degree of symptom expression with extreme susceptibility to deterioration. Preliminary trials have further indicated that the existing commercial varieties form a series between the extremes represented by their parent species (*F. chiloensis* and *F. virginiana*), some varieties, e.g., Lefebvre and Huxley's Giant approximating in reaction to the former, and others, e.g., Royal Sovereign and Sir Joseph Paxton, to the latter species."

From the above it would appear that the English workers have not yet encountered plants among the wild species or domestic varieties that show as high resistance to yellow-edge as do those of certain clones of *F. virginiana* indigenous to Ontario. It is intimated by Harris and King (5) that "samples of *F. indica* and *F. virginiana* (from North American sources)" are to be used in further tests as to their efficiency as indicator plants. It will be interesting to await the results of these tests, especially as regards *F. virginiana*, to see how they compare with those reported in the present paper.

Of interest in the present studies was the observance, first, of such wide variations in inherent vigour of growth among clones of the wild species and then, the discovery of the apparent correlation between vigour of vegetative growth and resistance and susceptibility to the disease. From investigations along other lines which have been carried out at St. Catharines during recent years, it was learned that *F. virginiana* plants are in general much more resistant to root rot than those of any of the cultivated varieties tested. These findings together with those reported in the present paper suggest implications of practical importance. In breeding new varieties of strawberries involving *F. virginiana* as one of the parents, it would seem highly desirable to employ clones of the latter, which, in addition to outstanding vigour of growth, possess the important character of resistance to root rot and to the virus of yellow-edge.

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

XI. SIMULTANEOUS RELATIONS BETWEEN MALT EXTRACT AND TWO OR MORE BARLEY PROPERTIES¹

BY H. R. SALLANS², W. O. S. MEREDITH³, AND J. A. ANDERSON⁴

Abstract

Inter- and intravarietal relations between malt extract and barley properties (extract, starch, total nitrogen, 1000-kernel weight, salt-soluble nitrogen, saccharifying activity, cellulose-lignin residue, and steeping time) have been investigated by developing prediction equations for malt extract.

The most useful single factors for intervariatal prediction are barley extract, starch, and cellulose-lignin residue, in the order given. The inclusion of salt-soluble nitrogen and steeping time, as additional independent variables, with barley extract or starch, results in a significant improvement in the level of prediction. The most accurate equation was: *malt extract* = $1.1 + 0.93 \text{ barley extract} + 7.44 \text{ salt-soluble nitrogen} - 0.035 \text{ steeping time}$. This equation serves to indicate the relative extract yield of varieties grown at the same station, the standard error being $\pm 0.8\%$. The constant, 1.1, varies from station to station; hence, although this average value gives relative extract yields, the constant must be evaluated for specific environments if absolute extract yields are required.

The most useful single factors for intravarietal prediction are barley extract, starch, and total nitrogen. Statistical analysis showed that only barley extract and total nitrogen could be effectively combined for prediction purposes. The equation is: *malt extract* = $A + 0.58 \text{ barley extract} - 2.4 \text{ total nitrogen}$, standard error $\pm 0.6\%$. The factor *A* is dependent on variety but the data suggest that for Canadian malting varieties this constant has a value of about 35.7.

Attempts to develop a generalized equation applicable to all samples, irrespective of varieties and the environment in which they were produced, proved unsuccessful. This is apparently due to significant differences between the inter- and intravarietal partial regression coefficients for the properties studied.

In previous papers in this series, the relations between malt extract and each of a number of barley properties were examined and the possibilities of predicting the former from data on one of the latter were discussed. In the present paper, the treatment of the data is expanded to include studies of the simultaneous relations between malt extract and two or more barley properties and of the possibilities of developing prediction equations containing more than one independent variable.

Data on 12 varieties of barley grown at 12 Canadian experimental stations in 1937 (1-5, 10-13) were used in the present study. In addition, the more interesting relations were re-examined in the light of an additional set of data (not yet published) for 24 varieties grown at six Canadian experimental stations in 1938.

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In considering the subject of prediction, certain conceptions must be kept clearly in mind. It is apparent that differences, with respect to any barley or malt property, that exist between samples of different varieties grown at the same station, result from genetic or hereditary dissimilarities whereas differences that exist between samples of the same variety (from different stations) result from dissimilarities in the environments under which the samples were grown. Moreover, it follows that such intervarietal associations as occur between any two properties are governed by hereditary factors and that intravarietal associations are governed by environmental factors. In general, when an association between two properties results from a fundamental and close relationship between these properties, this association will exist both within and between varieties and will be invariable irrespective of the individual varieties or environments represented by the samples from which the association is deduced. On the other hand, there exist certain associations between barley and malt properties that do not represent close and fundamental relations but appear to reflect complex interrelations between a number of properties. Associations of the latter type may exist within varieties but not between varieties, or vice versa, or may exist both within and between varieties, but in such a way that the inter- and intravarietal relations obviously differ in character. All these types of associations have been found in the present investigation and the necessity for making separate examinations of inter- and intravarietal correlations has thus been amply established. The work thus leads to the development of certain equations for the prediction of malt extract that can be applied only to samples of different varieties grown under the same environmental conditions, of other equations that can be applied only to samples of one variety, or closely related group of varieties (cf. 6-9), and finally to the study of general equations which are applicable to all barley samples irrespective of variety or origin.

Intervarietal Prediction Equations

In developing an equation for the prediction of malt extract based on two barley properties, it is logical to select, as one of the latter, a property that is closely associated with malt extract. The degrees of association between malt extract and the more important barley properties studied in the 1937 investigation (in which 12 varieties were grown at each of 12 stations) are shown by the simple intervarietal correlation coefficients given in the first column of data in Table I. An examination of these coefficients shows that the three barley properties that show the most promise for prediction purposes are barley extract, cellulose-lignin residue, and starch.

If barley extract is used as one property, the second property that can best be combined with barley extract, can be selected by considering partial correlation coefficients, independent of barley extract, between malt extract and each remaining barley property. These partial correlation coefficients are given in the second column of data in Table I. The remaining two columns

of data give the corresponding partial correlation coefficients, independent of starch and independent of cellulose-lignin.

TABLE I

INTERVARIETAL CORRELATION COEFFICIENTS BETWEEN MALT EXTRACT AND CERTAIN BARLEY PROPERTIES

Barley property	Correlation coefficient			
	Simple	Partial, independent of:		
		Barley extract	Starch	Cellulose-lignin residue
Total nitrogen	.350	-.039	-.084	-.195
Alcohol-soluble nitrogen	-.200	-.235	-.286	-.417
Insoluble nitrogen	-.728**	-.577	-.545	-.366
Acid-resistant nitrogen	-.816**	-.450	-.510	-.298
Cellulose-lignin residue	-.912**	-.267	-.624*	—
Starch	.854**	-.526	—	-.105
Barley extract	.14**	—	.776**	.291
Saccharifying activity	-.001	.316	.695**	.674*
Salt-soluble nitrogen	.452	.876**	.839**	.640*
Steeping time	-.589*	-.791**	-.859**	-.814**
1000-kernel weight	.439	-.464	-.398	-.374

NOTE: In this and later tables (*) indicates that a 5% level of significance and (**) that a 1% level of significance is attained.

Only two of the partial correlation coefficients between malt extract and barley properties, independent of barley extract, are significant. These are the coefficients for salt-soluble nitrogen and for steeping time. Accordingly, it would appear that the prediction of malt extract from barley extract can be improved by inclusion in the prediction equation of either salt-soluble nitrogen or steeping time. Since the remaining partial correlation coefficients in the second column of data are not significant, none of the other barley properties can be combined advantageously with barley extract for prediction purposes.

Inspection of the last two columns of data in Table I will show that for predicting malt extract, both salt-soluble nitrogen and steeping time also lend themselves best to combination with either starch or cellulose-lignin residue. Significant but lower partial correlation coefficients, independent of starch, are also given by malt extract and each of the following three barley properties:—cellulose-lignin residue, barley extract, and saccharifying activity. However, although the partial correlation coefficient between malt extract and cellulose-lignin residue, independent of starch, is significant, that between malt extract and starch, independent of cellulose-lignin residue is not significant. It would thus appear that the negative relation between malt extract and cellulose-lignin residue (i.e., the positive relation between malt extract and the acid-soluble portion of barley) is closer than the relation between

It extract and starch. This can readily be conceived since it is apparent that malt extract includes, in addition to the starch, certain other soluble and readily hydrolysable components of the barley which are removed by treatment with boiling acid. Thus, for purposes of predicting malt extract, there is no advantage in combining starch with cellulose-lignin residue as is shown by the fact that the partial correlation coefficient for malt extract and this residue, independent of starch, is not significant. Exactly the same situation exists with respect to the relations between malt extract, barley extract, and starch; no significant improvement in the prediction of malt extract from barley extract can be obtained by including starch as a second independent variable.

The associations between malt extract, barley starch, and the saccharifying activity of the barley are somewhat more complex. Whereas the simple correlation between extract and saccharifying activity is not significant, the partial correlation between these two properties, independent of starch, is significant. It thus appears that there is a direct association between extract and saccharifying activity, but this is masked in the simple correlation by the associations that exist between each of these properties and starch content. The statistics suggest that in developing an equation for the prediction of malt extract, it might be worth while to consider the simultaneous relation between extract, on the one hand, and starch and saccharifying activity, on the other.

Having selected those pairs of properties that seem most promising for prediction purposes, the multiple correlation coefficients for malt extract and each pair can now be examined. To these may be added certain multiple correlations involving three rather than two independent variables. The various correlation coefficients are listed in the first column of data in Table II. The next column shows the results of analyses of variance undertaken to determine whether the addition of each independent variable raises the correlation coefficient significantly.

The study of data on samples of 12 varieties grown at each of 12 stations in 1937 was supplemented with a further study of 24 varieties grown at 6 stations in 1938. In the latter study, only those barley properties and associations, that had appeared most useful in previous work, were re-examined. The resulting correlation coefficients, together with information on the significance of added variables, are given in the last two columns of Table II.

The 1937 data for variety means show that several pairs of barley properties are correlated with malt extract to about the same degree. These pairs include: (a) barley extract and salt-soluble nitrogen or steeping time and (b) starch and each of the two last named properties. In each case the multiple correlation coefficient is significantly higher than the corresponding simple coefficient. When salt-soluble nitrogen and steeping time are combined with barley extract, giving three independent variables, the slight improvement in the multiple correlation is not significant whereas, if starch is substituted for barley extract, the improvement in the multiple correlation coefficient attains the 5% level of significance.

TABLE II

INTERVARIETAL CORRELATION COEFFICIENTS BETWEEN MALT EXTRACT AND CERTAIN BARLEY PROPERTIES

Barley property	1937 data ¹		1938 data ²	
	Correlation coefficient	Significance of added variable	Correlation coefficient	Significance of added variable
Barley extract	.914**	—	854**	—
Barley extract × salt-soluble nitrogen	.980	**	919**	**
Barley extract × steeping time	.969**	**	906**	**
Barley extract × salt-soluble nitrogen × steeping time	.984**	0	944**	**
Starch	.854**	—	785**	—
Starch × saccharifying activity	.927**	*	826**	*
Starch × salt-soluble nitrogen	.959**	**	939**	**
Starch × steeping time	.964**	**	892**	**
Starch × salt-soluble nitrogen × steeping time	.976**	*	972**	**
Acid-resistant residue	— .912**	—	—	—
Acid-resistant residue × salt-soluble nitrogen	.939**	0	—	—
Acid-resistant residue × steeping time	.971**	**	—	—
Acid-resistant residue × salt-soluble nitrogen × steeping time	.972**	*	—	—

¹ 12 varieties grown at 12 stations in 1937.

² 24 varieties grown at 6 stations in 1938.

The multiple correlation coefficients for malt extract, starch, and saccharifying activity and for malt extract, cellulose-lignin residue, and other properties are somewhat lower and suggest that these various combinations are less useful for prediction purposes. It was for this reason that cellulose-lignin residues were not studied again in 1938.

In 1938, when 24 varieties comprising a wider range were used, the correlation coefficients proved to be somewhat lower, as might be anticipated. Among the correlations involving two independent variables, that for starch and salt-soluble nitrogen gave the highest coefficient whereas, for the 1937 data, the highest coefficient was given by barley extract and salt-soluble nitrogen. In 1938, as in 1937, the correlation between malt extract, starch, salt-soluble nitrogen, and steeping time, proved to be significantly higher than those correlations in which one of the last two variables was omitted. A similar situation existed in 1938 with respect to the corresponding correlations involving barley extract, although no improvement had been obtained by using a third independent variable in this series during the previous year.

As a result of these preliminary studies of data for two years, it appeared worth while to undertake a more exhaustive examination of the relations between malt extract and each of the following sets of barley properties:—

tract and salt-soluble nitrogen; extract and steeping time; extract, salt-soluble nitrogen, and steeping time; and the three corresponding sets of properties in which starch was substituted for barley extract.

Each of these relations between malt extract and a set of barley properties represents the equation of a surface. When two barley properties are involved, the equation takes the form:—

$$E = A + b_1P_1 + b_2P_2$$

When three barley properties are involved it becomes:—

$$E = A + b_1P_1 + b_2P_2 + b_3P_3.$$

In each of these equations E represents the dependent variable, malt extract; P values represent the various barley properties; and A and b values are constants, the former representing the E intercept and the latter the partial regression coefficients of malt extract on each barley property. For any given set of appropriate data the partial regression coefficients (b values) and the remaining constant A can be calculated by the usual statistical methods.

We may consider, as an example, the relation between malt extract, barley extract, salt-soluble nitrogen, and steeping time derived from the 1937 data. Since these data represent a study of 12 varieties grown at each of 12 stations, 13 intervarietal prediction equations for malt extract can be computed, namely, one equation for each of the 12 stations and an average equation for all stations combined. These equations were calculated and it was found that the partial regression coefficients (b values) and the values of the constant A differed slightly for each equation. It was therefore necessary to determine whether these differences were sufficiently small to be accounted for by the experimental errors of the investigation, or whether they were large enough to indicate that the relation is not homogeneous and differs from station to station.

This examination was made by analysing the residual variance into three portions:—(i) differences among station regression coefficients, representing that part of the residual variance due to differences in the slopes of the equation surfaces (b values) for each of the 12 stations; (ii) deviations of centroids from the average regression, representing that part of the residual variance due to differences in the positions of the equation surfaces for each of the 12 stations; (iii) deviations of the observations from each of the 12 individual regression functions, a remainder that may be used as an estimate of experimental error. The results of this analysis of residual variance are given in the first two columns of data in Table III. The statistics show conclusively that the centroids (i.e., the positions of the regression surface for each station) differ significantly, but that such differences as exist between the b values (i.e., the slopes of the partial regression surfaces) may well be due to experimental errors.

The second two columns of data in Table III show the results of similar analyses of residual variance made for the 1938 data. Exactly the same

TABLE III

ANALYSIS OF RESIDUAL VARIANCE FOR THE INTERVARIETAL RELATION BETWEEN MALT EXTRACT, BARLEY EXTRACT, SALT-SOLUBLE NITROGEN, AND STEEPING TIME

Variance due to:	1937 data		1938 data		Combined data	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Differences among station regression coefficients	33	0 542	15	0 785	51	0 748
Deviations of centroids from average station regression	11	2 866**	5	9 595**	17	5 038**
Deviations from individual station regressions	96	0 670	120	0 558	216	0 608

situation exists; the b values do not differ significantly but there are significant differences in the centroids from station to station.

The b values for the two years were compared by subjecting the combined data to an analysis of residual variance. The results of this analysis appear in the last two columns of Table III and show that the regressions are homogeneous. A comparison of the residual variance for differences among regression coefficients in each of the two years gave an F value of 1.45 as compared with 2.02 for the 5% level of significance. Hence no significant differences between the b values exist between years.

In terms of the prediction equation,

$$E = A + b_1P_1 + b_2P_2 + b_3P_3,$$

this means that whereas the constants b_1 , b_2 , and b_3 can be assumed to have the same values under all environments studied, the constant A should be given a different value for each environment. In other words, a general equation can be derived in which b_1 , b_2 , and b_3 are given definite values but A is not evaluated. This equation will serve for predicting the differences between any pair of varieties grown at the same station, or at the same set of stations, but it will not serve for the prediction of the actual yield of malt extract given by any variety grown at a certain station, unless the appropriate A value for that particular station is known.

For certain purposes this limitation is not important. Thus a plant breeder will generally wish to compare a new variety with a standard variety grown under the same conditions. Under these circumstances, it will be sufficient to know whether the predicted malt extract yield of the new variety is higher or lower than that of the standard variety and to what extent. Knowledge of the actual levels of malt extract predicted for the two varieties is of little importance. If it is known that a new variety will probably produce an average of 2% more extract than the standard variety, when grown at a certain set of stations, it is of little significance whether the actual values for the varieties turn out to be 74 and 72%, or 72 and 70%.

TABLE IV

ANALYSIS OF RESIDUAL VARIANCE FOR INTERVARIETAL PREDICTION OF MALT EXTRACT FROM EQUATIONS INVOLVING VARIOUS COMBINATIONS OF TWO OR THREE BARLEY PROPERTIES

Data	Variance due to:	Degrees of freedom	Mean square				Degrees of freedom	Mean square	
			Barley extract and:		Starch and:			Salt-soluble nitrogen, steeping time, and:	
			Salt-soluble nitrogen	Steeping time	Salt-soluble nitrogen	Steeping time		Barley extract	Starch
1937	Differences among station regression coefficients	22	0 644	0 832	0 521	1 126	33	0 542	0.572
	Deviations of centroids from average station regression	11	5 584**	3 281**	6 207**	4 099**	11	2 866**	5 144**
	Deviations from individual station regressions	108	0 691	0 623	1 026	0 795	96	0 670	0 723
1938	Differences among station regression coefficients	10	1 033	1 604*	2 374**	1 837	15	.785	1 693**
	Deviations of centroids from average station regression	5	8 659**	4 291**	25 419**	12 050**	5	9 595**	25 162**
	Deviations from individual station regressions	126	0 673	0 741	0 830	1 271	120	0 558	0 749

The various combinations of barley extract, starch, salt-soluble nitrogen, and steeping time listed in Table II were examined in a similar manner and the results of the analysis of residual variance are collected in Table IV. From the data it is evident that in all cases there are significant deviations of the centroids from the average regressions and consequently the factors *A* cannot be evaluated for the preparation of generalized equations applicable to all stations. However, for comparing varieties grown at the same station, or group of stations, in the same year, an average value of *A* may be used effectively. The data also show that the partial regression coefficients, or *b* values, are homogeneous within years for the relations between malt extract and the following barley properties: (*a*) barley extract and salt-soluble nitrogen; (*b*) starch, steeping time, and barley extract; (*c*) salt-soluble nitrogen and steeping time. Furthermore, it can be shown that the *b* values for these relations do not differ significantly between years. Thus these relations may be used in the preparation of intervarietal prediction equations valid for both the 1937 and 1938 data.

Intervarietal prediction equations are listed below. With the exception of the sixth, these are based on two years' data representing 288 samples; the final equation involves cellulose-lignin residue that was derived from the 1937 data and represents 144 samples. The standard error of prediction for a single sample is given in parentheses after each equation. In general, it will not prove satisfactory to test new varieties by growing them at one station only, owing to the fact that they do not always fall in exactly the same order

with respect to malt extract when grown at different stations. To overcome this difficulty the authors recommend growing the varieties at a minimum of four stations to obtain reasonably accurate estimates of potential yields of malt extract. If this is done the standard error of estimate of the variety means will become approximately one-half the value indicated after each equation.

INTERVARIETAL PREDICTION EQUATIONS

$$\begin{aligned}
 E &= 1.0 + 0.96 B & (\pm 0.98) \\
 E &= -4.0 + 0.94 B + 10.4 S.N & (\pm 0.86) \\
 E &= 1.1 + 0.93 B + 7.44 S.N - 0.035 S.T & (\pm 0.80) \\
 E &= 25.5 + 0.89 S & (\pm 1.21) \\
 E &= 29.7 + 0.90 S - 0.067 S.T & (\pm 1.07) \\
 E &= 49.9 - 2.02 C & (\pm 1.25)
 \end{aligned}$$

In the above equations, E = malt extract, B = barley extract, $S.N$ = salt-soluble nitrogen, $S.T$ = steeping time, S = starch, and C = cellulose-lignin residue.

Intravarietal Prediction Equations

The simple intravarietal correlation coefficients for malt extract and various barley properties are given in the first column of data in Table V. Examination of these will show that malt extract is most highly correlated with barley extract, starch content, total nitrogen, and alcohol-soluble nitrogen. In presenting the partial correlation coefficients given in the remaining columns of the table, those independent of alcohol-soluble nitrogen have been left out since they are essentially similar to corresponding coefficients independent of total nitrogen.

Superficially it might appear that starch could be combined with barley extract but since the correlation between malt extract and starch, independent

TABLE V

INTRAVARIETAL CORRELATION COEFFICIENTS BETWEEN MALT EXTRACT AND CERTAIN BARLEY PROPERTIES

Barley property	Correlation coefficient			
	Simple	Partial, independent of:		
		Barley extract	Starch	Total nitrogen
Total nitrogen	-.957**	-.755**	-.453	—
Alcohol-soluble nitrogen	-.958**	-.835**	-.571	-.223
Insoluble nitrogen	-.883**	-.416	-.199	.022
Acid-resistant nitrogen	-.940**	-.711*	-.103	.119
Cellulose-lignin residue	-.329	.380	.188	-.569
Starch	.967**	.501	—	.625*
Barley extract	.973**	—	.622*	.854**
Saccharifying activity	.861**	-.682*	-.331	.037
Salt-soluble nitrogen	.767**	-.304	.046	.306
Steeping time	.722**	-.524	-.289	.503
1000-kernel weight	.600*	-.412	-.349	.416

of barley extract, is not significant, it is apparent that the combination of starch with barley extract will not prove advantageous.

A similar situation exists with respect to the combination of total nitrogen with starch. The partial correlation independent of total nitrogen is significant but that independent of starch is not. Consequently the combination will not prove useful. The only other partial correlation, independent of total nitrogen, which is significant, is that between malt extract and barley extract. Accordingly, as previously noted, the prediction of malt extract from barley extract and total nitrogen will prove to be significantly better than the prediction from either factor alone.

TABLE VI
INTRAVARIETAL CORRELATION COEFFICIENTS BETWEEN MALT EXTRACT AND CERTAIN
BARLEY PROPERTIES

Barley property	Correlation coefficient	Significance of added variable
Barley extract	.973**	—
Barley extract \times total nitrogen	.989**	**
Barley extract \times acid-resistant nitrogen	.982**	0
Barley extract \times saccharifying activity	.986**	*
Starch	.967**	—
Total nitrogen	— .957**	—
Total nitrogen \times 1000-kernel weight	.964**	0

The multiple correlation coefficients for malt extract and those combinations of factors discussed above, are given in Table VI. The correlation coefficient for malt extract, total nitrogen, and 1000-kernel weight is also included since it has previously been examined by Bishop (6-8). The last column in Table VI shows the results of analyses made to determine whether the addition of the second independent variable raised the correlation coefficient significantly.

Inspection of the table will show that a significant increase in the correlation coefficient for malt extract and barley extract occurs when total nitrogen or saccharifying activity is added as a second independent variable. The influence of saccharifying activity on this relation is apparently due to the

TABLE VII
ANALYSIS OF RESIDUAL VARIANCE BY VARIETIES FOR PREDICTION OF MALT EXTRACT FROM THE
MULTIPLE FACTORS TOTAL NITROGEN AND BARLEY EXTRACT

Variance due to:	Degrees of freedom	Mean square
Differences among varietal regression coefficients	22	0.46437
Deviations of centroids from average varietal regression	11	9.02368**
Deviations from individual varietal regressions	108	0.33224

close intravarietal association between saccharifying activity and total nitrogen (3, 9). Consequently, only the relation involving total nitrogen is considered in the following discussion. An analysis of residual variance, given in Table VII, shows that for this relation there are significant differences in the centroids for different varieties but not in the regression coefficients.

The prediction equation is as follows:—

$$\text{Malt extract} = A + 0.58 \text{ barley extract} - 2.4 \text{ total nitrogen } (\pm 0.6).$$

The constant A has the following values for the different varieties studied:—

O.A.C. 21	35.7	Nobarb	33.8	Charlottetown	36.0
Mensury	35.8	Regal	33.8	Hannchen	36.1
Olli	36.6	Wisconsin	33.4	Victory	35.4
Peatland	35.1	Velvet	34.8		
Pontiac	34.8				

It will be observed that these values range from 36.6 for Olli to 33.4 for Wisconsin, so that if an attempt were made to use the same value for each variety a considerable error would be introduced. However, certain pairs of varieties have closely similar values. These include:— the two closely related rough-awned six-rowed varieties, O.A.C. 21 and Mensury; the two smooth-awned six-rowed varieties, Nobarb and Regal; and the two-rowed varieties, Charlottetown 80 and Hannchen.

Since O.A.C. 21 and Mensury make up the bulk of the six-rowed barley now sold for malting barley in Canada, it would appear that a satisfactory equation for prediction of the extract yield of commercial shipments of Canadian barley may be developed. Our data suggest that this will take the form:

$$\text{Malt extract} = 35.7 + 0.58 \text{ barley extract} - 2.4 \text{ total nitrogen}$$

However, as has been pointed out by Bishop (6, 7), the value of the varietal constant is affected by malting conditions. Thus our value, 35.7, based on studies of malts made in small-scale laboratory equipment, may not prove accurate for malts made in commercial malt houses.

General Prediction Equation

The possibilities of developing a general intervarietal prediction equation applicable at all stations appeared to merit investigation. For this purpose it is necessary to combine in one equation factors showing intervarietal and interstation relations with malt extract. Preliminary investigation suggested that the three properties, barley extract, steeping time, and total nitrogen were most promising. The following equation was developed from the 1937 data:—

$$\begin{aligned} \text{Malt extract} = & 16.0 + 0.849 \text{ barley extract} - 0.043 \text{ steeping time} \\ & - 1.54 \text{ total nitrogen } (\pm 0.86) \end{aligned}$$

The results of an analysis of the homogeneity of the relation, with respect to stations, are given in the first two columns of data in Table VIII. Significant differences between station regressions and between centroids were not established and the equation therefore seemed promising.

A reinvestigation of this equation was made with the 1938 data. The results of the analysis are shown in the last two columns of data in Table VIII. Once again our purpose was defeated since the analysis shows that for the 1938 data differences between centroids are significant. Accordingly, even in this equation, it would appear that separate constants for different stations should be employed.

TABLE VIII

ANALYSIS OF RESIDUAL VARIANCE FOR INTERVARIETAL RELATION BETWEEN MALT EXTRACT, BARLEY EXTRACT, STEEPING TIME, AND TOTAL NITROGEN

Variance due to:	1937 data		1938 data	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Differences among station regression coefficients	33	0 661	15	0.834
Deviations of centroids from average station regression	11	1 322	5	5.660**
Deviations from individual station regressions	96	0 729	120	0.738

A number of modifications of this equation were examined without success. Significant differences were invariably found between centroids. The authors consider it probable that adequate analyses of a sufficiently large body of data will invariably show that all general equations for the prediction of the malt extract yield of any sample of barley, irrespective of variety and origin, are theoretically unsound. It does not follow that a useful general equation cannot be developed but this will probably be based on compensating errors rather than on sound principles.

Discussion

The most useful intervarietal prediction equations for malt extract are based on the use of either barley extract or starch content. The explanation is readily apparent since it is obvious that a reasonably close relation must exist between malt extract and each of these two properties. Except for small portions, lost by respiration during malting and retained in the spent grains during mashing, all the starch in the barley is transformed into malt extract of which the starch degradation products form the predominant part. Similarly, barley extract, as determined in the present investigation, includes almost all of the barley constituents that are later transformed into extract, together with those that are lost during malting by steeping loss, respiration, and removal of sprouts. A close and fundamental relation between barley extract and malt extract would therefore be anticipated.

In effect it would appear that the determinations of barley extract provide a measure of the potentially extractable material in the barley kernel. Moreover, since the starch forms such a large proportion of this material, it also provides a measure of the potential extract. Thus both of these properties

provide a logical starting point for the development of a prediction equation for malt extract.

Having obtained a factor that measures the potential yield of malt extract, it would appear that the second factor required for the prediction of the actual yield should measure the degree to which the potential yield can be realized during the malting and mashing processes. This factor is probably associated with enzymatic activity and it can be assumed that the higher the enzymatic activity of the variety, the greater will be its yield of extract. However, two opposing reactions are involved. Increasing enzymatic activity will undoubtedly result in hydrolysis of increasing amounts of potentially extractable material. At the same time it also appears that increasing enzymatic activity will also result in increasing the malting loss resulting from the activity of the respiratory enzymes and the ready availability of soluble material for translocation to the roots which are subsequently discarded when the malt is polished. So far as can be determined from the results of the present investigation, the balance between these opposing reactions is favourable. Thus though increasing enzymatic activity results in a slight increase in malting loss, this appears to be more than offset by the additional hydrolysis of potentially extractable material. In this connection, it is interesting to note that an intervarietal association exists between malting loss and extract yield so that varieties that tend to have a higher malting loss also tend to produce a greater yield of extract.

The practical difficulty is that of discovering a barley property that provides an adequate measure of the total enzymatic activity of the grain. In this connection a determination of the saccharifying activity of a barley extract made in the presence of papain has been investigated (10). A fairly close intervarietal relation exists between this activity and the saccharifying activity of the finished malt. Moreover, between varieties, the saccharifying activity of the barley is associated to some extent with starch liquefying activity, autolytic diastatic activity, and proteolytic activity, which were the only other enzymatic properties investigated in this study (4, 11). Thus varieties that tend to be high in saccharifying activity of barley also tend to be high in proteolytic activity, autolytic diastatic activity, and starch liquefying activity of the malt. It would thus appear that saccharifying activity of barley might form an estimate of the total enzymatic activity developed during malting. Statistical studies show that this expectation could not be realized, although an improvement in the prediction of malt extract from starch content could be obtained by adding barley saccharifying activity as a second independent variable.

Although it is possible to obtain an estimate of the saccharifying activity of malt by making a determination of the saccharifying activity of the barley by means of a papain extract, there are not yet available, so far as we are aware, similar methods of estimating other enzymatic activities of malt. In the present study, the starch liquefying activity of the malt was measured directly. According to current hypothesis this activity is related to the content

α -amylase and appears to be developed during the malting process. In consequence, it seems impossible to obtain an estimate of the starch-liquefying activity of malt by any type of determination made on the barley. In this study the proteolytic activity of the malt by an autolytic procedure was also determined. Although proteolytic activity increases very markedly during the malting process, the barley has some proteolytic activity prior to malting. Preliminary experiments, made to determine whether the proteolytic activity of the barley was related in any way to the proteolytic activity of the malt made from it, failed to suggest that any such relationship existed. The subject would appear to merit further investigation but must await the development of more satisfactory methods of estimating the activities of the various proteolytic enzymes.

Further statistical studies (11) indicated that the relations between barley saccharifying activity and the enzymatic activities of malt do not appear to represent fundamental relations. All these enzymatic activities are positively associated with salt-soluble barley nitrogen, and when the partial correlations between pairs of activities, independent of salt-soluble nitrogen, are computed, the coefficients fail to attain the 5% level of significance. It thus appears that the more fundamental relations involved are those between salt-soluble nitrogen and the enzymatic activities. Although these relations are not sufficiently close to permit accurate prediction of enzymatic activities of malt from the salt-soluble nitrogen of the barley, nevertheless, they suggest that a real relation exists between salt-soluble protein, or some fraction of it, and the enzymes present in barley and malt. Either the enzymes themselves are similar in nature to the salt-soluble proteins or these proteins act as carriers for the enzymes. It appears that salt-soluble barley nitrogen may well be a property that tends to represent total enzymatic activity of malt.

Confirmation of this hypothesis was obtained by a significant improvement in the prediction of malt extract when salt-soluble nitrogen was introduced as a second independent variable in equations involving barley extract or starch. In equations with starch and salt-soluble nitrogen an alternative hypothesis is possible. Since malt extract is composed largely of the hydrolytic products of starch and protein, it is not unreasonable to suppose that the salt-soluble protein may make a major contribution to the latter of these components. However, in equations involving barley extract it appears that the barley extract itself includes the major portion of the salt-soluble nitrogen in addition to the starch. Hence in these equations the beneficial effects of including salt-soluble nitrogen must depend on less direct relations, presumably those between this property and enzymatic activities.

A second property that has proved useful, in combination with barley extract or starch, for the prediction of malt extract, is steeping time. In the present investigation this represents the number of hours required for a barley to attain a moisture content of 46% when steeped under specified conditions of temperature and aeration. It is apparent at once that the usefulness of this property as a second independent variable must depend upon indirect

relations. Here again we are of the opinion that the relations in question are those between steeping time and individual enzymatic activities. Thus it has been shown that the intervarietal correlations between steeping time on the one hand, and saccharifying activity of barley, saccharifying activity, proteolytic activity, autolytic diastatic activity, and starch liquefying activity of malt on the other hand, all attain a 1% level of significance. The relations are all inverse so that varieties that tend to require a longer time in the steep tend to have lower enzymatic activities. The authors of this paper are not able to offer any satisfactory hypothesis to explain these associations. However, in this connection it should also be noted that there is a negative association between steeping time and salt-soluble nitrogen. Partial correlation coefficients, independent of steeping time, between the various pairs of enzymatic activities showed a close similarity to those independent of salt-soluble nitrogen. Hence it is concluded that the effectiveness of steeping time, in improving the prediction of malt extract from barley extract or starch, results from the associations of this property with the various enzymatic activities of the malt.

The investigation also showed that the intervarietal prediction of malt extract could be improved by including both salt-soluble nitrogen and steeping time with barley extract or starch. Here again the explanation appears to be that a combination of salt-soluble nitrogen and steeping time provides a better estimate of total enzymatic activities than either of these properties alone.

Turning now to the intravarietal (or interstation) prediction of malt extract, a rather different situation is found. As might be expected, barley extract still proves to be the most useful individual property for purposes of predicting malt extract. Again, we may assume that its utility depends primarily on the fact that it measures potentially extractable material. The same reasoning also appears to apply to the use of total nitrogen as a prediction factor. Its utility has been widely investigated by Bishop who suggests that it provides an inverse measure of the potentially extractable carbohydrate material (8).

Following the line of reasoning used in discussing the intervarietal prediction of extract, it would appear that in intravarietal prediction a search should also be made for a second factor representing the degree to which the potentially extractable material can be made available. Certain obvious difficulties are at once faced. It has been demonstrated conclusively that a change in environment, which results in an increase in barley extract or starch, also results in a decrease in total nitrogen content (13) and, with the exception of autolytic diastatic activity (3, 4, 11), of all enzymatic activities so far investigated. Thus in considering the effect of environment on malt extract we are dealing with two opposing tendencies, an increase in potentially extractable material associated with a decrease in enzymatic activities. A third factor may also play a part in this connection. It is quite possible, for instance, that the degree to which potentially extractable material can be realized during the malting;

and mashing process depends not only on the activity of enzymes involved but also on the facility with which the extractable material can be attacked by these enzymes. Bishop (8) has put forward an hypothesis in this connection in which he suggests that a decrease in malt extract is associated with an increase in total nitrogen content not only because an increase in proteins must result in the displacement of extractable carbohydrates but also because there is a tendency for some potentially extractable carbohydrates to be "locked up" by the proteins.

The only factor we have discovered that will improve the intravarietal prediction of malt extract from barley extract, is total nitrogen. Since in the prediction equation the partial regression coefficient for total nitrogen on malt extract is negative, there is no reason to believe that the improvement resulting from the inclusion of total nitrogen as a second independent variable is related to the association that exists between total nitrogen and enzymatic activities. If this hypothesis were correct, the partial regression coefficient should be positive rather than negative. Accordingly, it appears that in considering this relation between malt extract on the one hand, and barley extract and total nitrogen, on the other hand, we are forced to conclude that the effect of the last named factor is related to the degree in which total nitrogen "seals up" potentially extractable barley materials.

As was pointed out earlier in this paper, there appears to be little possibility of developing a general equation that will be satisfactory for both the inter- and intravarietal prediction of malt extract. Such an equation can only be developed if two sets of conditions prevail. Firstly, if it is possible to find factors such as barley extract and starch which are related to malt extract both within and between varieties, and secondly, if the regressions of these properties on malt extract are identical both within and between varieties. It has not been possible to find any properties that meet the second of these conditions. The inter- and intravarietal regression coefficients invariably differ appreciably. This might well be expected since no single barley property has yet been found that is sufficiently closely related to malt extract to provide adequate prediction by itself. Malt extract may be dependent principally upon the potential yield of extractable materials existing in the barley as measured by barley extract or starch, but it is also conditioned by a variety of other factors that appear to act differently within and between varieties. In consequence, the regressions of malt extract on barley extract or starch are not identical within and between varieties. Theoretically, it is quite possible that two or three barley properties might be found that are related to malt extract both in an inter- and intravarietal manner. If a prediction equation based on these properties were then developed it might finally appear that the partial regressions of each property on malt extract were identical both within and between varieties. It must be admitted, however, that our practical attempts to solve this theoretically soluble problem have not proved successful.

In surveying the results of the study presented in this paper, the authors are forced to admit that they do not expect that wide use will be made of the

various prediction equations discussed above. Prediction equations are, after all, only means of expressing the relations that exist between various barley properties and malt extract. They do not fail to be useful merely because they are not used. They may well serve to bring to light the various associations and more fundamental relations that exist between barley and malt properties, and may thus help to elucidate the nature of malting quality in barley. A clear conception of underlying principles is surely a necessary prerequisite to the development of a logical program designed to produce new varieties of improved agronomic quality and satisfactory malting quality.

The practical advantages that arise from a detailed knowledge of the relations between malt extract and barley properties are two, and these both depend upon an extensive knowledge of the chemical characteristics of the parent materials used in the breeding program. Firstly, it should be possible to select pairs of parents that are complementary in that they are not characterized by the same faults. Secondly, it should be possible, usually by the use of a single determination made on a small amount of barley, to select from the progeny of certain crosses those lines that are not characterized by an important malting deficiency. In Canada, close co-operation already exists between plant breeders and those engaged in laboratory studies of malting quality. It is hoped that as a result of the studies reported in this series of papers, laboratory workers will now be in a position to give more extensive and useful aid to plant breeders.

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STUDIES ON FILM-FORMING YEASTS¹

I. MEDIA AND METHODS

BY V. E. GRAHAM² AND E. G. HASTINGS³

Abstract

The methods used for the isolation and study of film-forming yeasts are described. Two of these methods are of special interest, viz., the observation of the structure of pseudomycelium on "cross-hatched" plates and the preparation of gypsum cultures in test tubes.

Introduction

Certain yeast-like organisms produce a film of varying thickness on the surface of liquid media and on foods in which ethyl alcohol and some of the common organic acids are present in moderate concentration. Because of their film-forming character such organisms are often referred to as "Mycoderma". This term has, however, been used as a generic name for many years, and a continuation of its use as a descriptive term leads to confusion. In this work the term "film-forming yeasts" is used in referring to the group as a whole and the term *Mycoderma* is used only in referring to the organisms belonging to this genus as described by Lodder (6).

In this study, film-forming yeasts have been isolated from rennet brine, Swiss cheese brine, ensilage, butter, and pickles. This paper deals with the methods that were used in the study of these organisms. The types found in the products mentioned will be discussed in later reports.

The literature dealing with the classification of the film-forming yeasts has been summarized elsewhere (3). This literature is voluminous but much of it is now only of historical interest. The nomenclature followed in this study is essentially that of Stelling-Dekker (8), Lodder (6), and Diddens and Lodder (2).

Media and Methods

Most of the methods used in this study of film-forming yeasts are familiar bacteriological and mycological procedures and will not be discussed in detail. The methods used for isolation and for the study of carbon and nitrogen sources will be presented, together with some observations on the problems of pseudomycelium formation and spore formation.

Isolation of Film-forming Yeasts

Film-forming yeasts may be isolated directly by ordinary plating on acid wort or malt agar when they constitute an appreciable proportion of the flora in the substance in which they occur. The enrichment procedure is the

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more generally useful one and was used in this work prior to plating. The composition of three useful enrichment media is given below.

Enrichment Medium No. 1

Bacto peptone	0.5%
Absolute alcohol	0.5%
Sodium chloride	0.5%

The reaction was adjusted to pH 4.6 by the addition of lactic acid.

Enrichment Medium No. 2

Magnesium sulphate	0.02%
Dipotassium phosphate	0.10%
Bacto peptone	0.50%
Glacial acetic acid	0.40%

The reaction was not adjusted.

Enrichment Medium No. 3

Ammonium sulphate	0.25%
Dipotassium phosphate	0.05%
Magnesium sulphate	0.01%
Bacto peptone	0.40%
Absolute alcohol	1.00%

The reaction was adjusted to pH 3.5 by the addition of lactic acid. The alcohol was usually added before sterilization but the concentration may be more closely regulated if it is added afterwards.

The media were placed in 6-oz. bottles to a depth of about 5 cm. and were plugged with cotton to allow free access of air. They were sterilized in the autoclave at 15 lb. pressure for 25 min. The medium was inoculated with some of the material from which the isolation of film-forming yeasts was sought. When a film had formed, a portion of it was transferred to another bottle of similar medium. If another film formed and microscopic examination showed the presence of yeast-like organisms in large numbers, loop dilution plates were prepared on acid glucose agar and the isolation and purification of the organisms was completed in the usual manner.

The medium containing acetic acid was devised for the isolation from soil of yeasts that could utilize this acid and also to aid in controlling mould growth. Medium No. 3 proved to be more generally useful than the others but No. 1 was used successfully in the isolation of *Debaryomyces* from rennet brine.

The principle of enrichment is very useful in securing cultures of film-forming yeasts. A variety of media can be devised for this purpose depending upon the growth requirements of the group being sought. Liquid cultures should not be disturbed; shaking causes the film to sink and tends to inhibit the growth of film-forming organisms.

Nitrogen Sources

Liquid media were used in studying the availability of nitrogen from ammonium sulphate, potassium nitrate, and peptone. The basic medium was that used by Stelling-Dekker (8), viz.:

Glucose	2.00%
Monopotassium phosphate	0.10%
Magnesium sulphate	0.05%

The nitrogen sources were added in the following proportions by weight: ammonium sulphate, 0.25%; potassium nitrate, 0.10%; peptone, 0.5%. Controls were used from which the nitrogen source was omitted. Distilled water must be used in preparing these media.

Carbon Sources

A determination of the materials that can be used as sources of carbon is often useful in classifying micro-organisms. The ability of an organism to secure carbon from a particular compound can be determined readily if it can utilize inorganic salts as nitrogen sources. For this purpose the following basic medium gave satisfactory results.

Monopotassium phosphate	0.10%
Magnesium sulphate	0.05%
Ammonium sulphate	0.25%

The carbon sources studied were added to this basic medium in the following proportions by weight: glucose, 1 or 2%; sucrose, lactose, and maltose, 1%; valeric, caprylic, and capric acids, 0.5%; butyric and caproic acids, 1%; maleic and tartaric acids, 0.33%; citric acid as 0.5% sodium citrate. In studying growth on ethyl alcohol 4% by volume was added after sterilization. All media were sterilized by autoclaving at 15 lb. pressure for 25 min.

When an organism cannot use inorganic compounds as nitrogen sources, peptone must be added to the medium. Since there is then growth on the control tubes, this somewhat complicates the study of carbon sources. In such cultures the decision regarding utilization was based on observation of the relative abundance of film formation on the control tube and the tube containing the added sugar or acid, and also by the final acidity or alkalinity. If only the peptone was used as a source of carbon the medium usually became alkaline, whereas if the sugar or other source of carbon was consumed, some acid was usually produced.

Stelling-Dekker (8) and Lodder (6) restrict the use of the term "fermentation" to the utilization of a sugar with the production of gas. "Utilization", on the other hand, was used to indicate that the organism consumed the sugar as a carbon source, but without gas production. In this work these terms are used in the limited sense.

Other Media

Cultures were carried on yeast water agar containing 2% glucose. Wort and malt agar are also satisfactory for this purpose and were used to some

extent. These and other media to which reference is made are commonly known and will not be described.

The Detection of Pseudomycelium


Lodder (6) has defined a true mycelium as one that is non-septate, or one in which the septa have been formed *within* the filament; a pseudomycelium on the other hand, is one that has been formed by the budding of its member cells, the septa thus being formed from the outside. This distinction is reasonably clear, although there are fungi in which both types of mycelium may be present. Pseudomycelium formation is an important character to be considered in the classification of yeast-like organisms and the conditions that favour its production have been studied in detail by many workers. It is important to recognize that the firmness with which the cells constituting the pseudomycelium are held together varies with different yeasts. Consequently, if the structure of the pseudomycelium is to be observed accurately, the method employed should be one in which the cells may be seen in the position in which they have developed. These facts explain the popularity of the slide culture technique of Rivalier and Seydel (7) and its various modifications (3).

It was found in the present study that pseudomycelium formation may be observed readily on an ordinary cross-hatched plate. Such plates were prepared by pouring 2% glucose yeast water agar in Petri dishes to a depth of about 6 mm. and inoculating by crossed streaks about 2 cm. apart as soon as the agar had hardened. The pseudomycelium grows out on the moist plate without distortion. The growth on such a plate is most abundant at the ends of the streaks and toward the periphery of the dish; it is least abundant toward the centre. Pseudomycelium formation is readily seen along the edges of the inner streaks in which growth is restricted. A detailed study of strands of the pseudomycelium may be made by placing mineral oil on the agar at the edge of a streak and, as described by Dalmau (1), examining directly with the oil immersion objective. This method requires no special medium or equipment and gives excellent results.

Spore Formation

The formation of ascospores is one of the most valuable characters to be considered in the classification of yeasts and many methods have been described for inducing these organisms to form spores. A study of the results of many workers indicates that the requirements for sporulation are not necessarily the same for all genera. As a rule the conclusion of Heinz (5) that conditions that favour vegetative growth tend to inhibit spore formation seems to be true; an exception, however, is found in at least one species of *Pichia*.

Three of the most commonly used methods for the detection of spore formation are: cultivation on gypsum blocks, carrot slices, and Gorodkova agar. All these methods were used in the present study. Gorodkova agar

 has given excellent results in studies on the genera *Hansenula* and *Debaryomyces*.

A New Method of Preparing Gypsum Cultures

Preparing gypsum cultures by Hansen's method (4) involves the expenditure of much time and labour; also the cultures tend to dry out and become contaminated. When it became necessary to use gypsum cultures in rather large numbers in this study the idea presented itself of making gypsum slants in ordinary test tubes. Preliminary experiments demonstrated that this procedure was feasible and that, apart from eliminating much of the work and difficulty ordinarily involved in the preparation of such material, the method was also easier and the tubes were less liable to contamination than dish cultures. The procedure developed is as follows. Procure a sufficient number of clean test tubes, preferably of the 18 by 150 mm. size. Set up a filling funnel equipped with rubber tubing and a stopcock but without a glass tip on the filling tube. The end of the tubing should be long enough to reach to the bottom of the test tube. Mix gypsum with water in such proportions that the mixture is of the consistency of thick cream. Fill the tubes in the ordinary manner but run the end of the filling tube down to within an inch of the bottom of the test tubes. If the gypsum has been mixed to the proper consistency there will be no drip from the end of this filling tube when the pinchcock is closed and thus there will be no drops of gypsum on the side of the test tube. Fill the tubes to a depth of about 1 in., place in suitable racks, and slope them to give a long surface for inoculation. Place the rack in this sloping position in the incubator at about 50° C. to harden. This will require 24 to 48 hr. When the slopes are hard, remove from the incubator, plug the tubes with cotton, and sterilize in the autoclave. Thorough hardening is important; if the gypsum is not properly dried, it will swell during autoclaving, owing to the formation of steam within the mass, and the tubes are useless.

Gypsum slopes prepared in this manner will keep indefinitely if given proper protection. When a culture is to be prepared, some moisture must be added to the tubes. This may be in the form of sterile water, wort, phosphate solution, or any other type of nutrient desired. About 3 ml. of the solution is usually required.

The tubes may be cleaned by filling with water, allowing to soak overnight, and then breaking the block loose from the tube with a small rod. The water tends to creep between the gypsum and the glass and the whole block usually slips out readily.

These tubes are handled in exactly the same manner as agar slants and have many obvious advantages over the usual type of dish culture. This method makes gypsum culture feasible on a large scale, either for research or teaching purposes.

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VEGETATIVE PROPAGATION OF CONIFERS

IX. EFFECTS OF CHEMICAL TREATMENTS AND A WAX SPRAY ON THE OUTDOOR PROPAGATION OF SPRUCE CUTTINGS¹

BY N. H. GRACE² AND J. L. FARRAR³

Abstract

Norway spruce cuttings were collected at intervals throughout the year, subjected to treatment with talc dusts containing from 5 up to 10,000 p.p.m. of indolylacetic acid, and propagated in outdoor frames. In some experiments, indolylacetic acid treatments were included in a series of dusts involving cane sugar, potassium acid phosphate, and organic mercurial disinfectants. Effects of wax coating of cuttings of Norway and white spruce and eastern white cedar were also considered.

Indolylacetic acid treatment failed to have any general beneficial effects on rooting; concentrations of 8000 and 10,000 p.p.m. were usually injurious. However, treatment of apparently dormant cuttings, taken shortly before emergence of new growth, with 1000 p.p.m. gave 25% rooting, as compared to 8% for the controls, and tended to increase the length of root. Treatments with organic mercury, cane sugar, and potassium acid phosphate increased survival of new growth, and, in combination with indolylacetic acid, increased survival and root lengths.

Talc treatment increased rooting to as much as 70% for cuttings planted in sand as compared to 25% for the controls, but had no effect on plantings in a sand-peat mixture. Talc effects were the most marked on cuttings taken before emergence of new growth and when planting was delayed for 24 hr. after treatment.

Wax application had no effect on dormant spruce cuttings but was markedly injurious to summer collections. Injurious effects were reduced when wax was used in conjunction with indolylacetic acid treatment.

Introduction

The effects of a number of chemical treatments on the responses of conifer cuttings have been reported (2-4, 6, 7, 9, 10, 14-20, 22, 24, 25, 29, 32). Early communications by the authors dealt with greenhouse propagation (3, 4, 6, 7, 9, 10, 14, 18). Since most applications of vegetative propagation to forestry necessitate large-scale operations, the use of outdoor frames has been given some consideration (5, 14, 17). This paper describes the effects of chemical treatments on cuttings propagated outdoors as shown by 16 experiments involving 16,650 cuttings.

Experimental

MATERIALS

All spruce cuttings were from 6 to 10 cm. long and were made of the full length of the current year's growth, in most cases from the lower branches of the tree. Norway spruce (*Picea Abies* (L.) Karst.), cuttings were obtained from a plantation 19 years old at the Petawawa Forest Experiment Station,

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Chalk River, Ontario. Collections were taken at monthly intervals from November to April, at several periods in May and June just prior to, and during, the emergence of new growth, and at bimonthly intervals from July to October.

Cuttings of the winter collections, in groups, were heeled in and put outdoors in flats till May when they were lined out in the frames. Those of the other collections were planted outdoors directly. Some experiments were done in sand and the others in sand-peat.

Effects of a wax emulsion were determined on white spruce (*Picea glauca* (Moench) Voss.) and eastern white cedar (*Thuja occidentalis* L.) as well as Norway spruce. These cuttings were obtained in Ottawa from planted trees about 15 years old. Spruce cuttings were collected while apparently dormant, about the time of emergence of new growth, and at intervals during the summer. Cedar cuttings were taken early in May while still dormant.

TREATMENT OF CUTTINGS

The chemicals were applied to the cuttings in finely ground talc, since the authors have found the solution method of treatment to be injurious or without effect (3, 4, 14, 18). The basal two centimetres of the cuttings were dusted without preliminary moistening and planted within two hours, except in one case where delayed planting was being investigated.

A phytohormone chemical was used in all experiments. Indolylacetic acid was selected because some beneficial effects on conifer cuttings had already been obtained through its use (6, 10, 25, 32). It was mixed with talc in concentrations of 5 to 10,000 p.p.m. (parts of the chemical per million parts of the mixture by weight), though in most experiments the concentrations were 5, 100, 1000, and 2000 p.p.m.

Most experiments were of a factorial design permitting the examination of interaction effects between phytohormone treatments and other factors such as the developmental stage of the tree, the position of the cutting on the tree, type of cutting, delayed planting, and media.¹ Only one or two of these factors were combined with indolylacetic acid treatment in any one experiment. Groups of untreated and talc treated cuttings were used as controls.

Nutrient and disinfectant chemicals were used in two experiments which are numbered and described in detail because they relate to a series of experiments dealing with the effects of relatively complex dust mixtures on cuttings of Norway spruce and other plants (8, 10, 13, 17).

Experiment 1

Cuttings of Norway spruce were collected from both the upper and lower thirds of the tree, May 17, 1939, while the buds were still dormant, and were treated in groups of 12 with a series of eight dusts (10). The series involved

¹ The primary effects of these factors will be reported in later papers.

indolylacetic acid at concentrations of 0 and 1000 p.p.m. alone, and in combination with cane sugar at 0 and 10% and ethyl mercuric phosphate at 0 and 10 p.p.m. There were four replicate blocks in the experiment making a total of 768 cuttings.

Experiment 2

Cuttings of Norway spruce bearing an opening bud with a new shoot ranging from 0.5 to 1 cm. in length, were collected from the lower third of the tree on June 9, 1939. Groups of 10 cuttings were treated with a series of 32 dusts containing indolylacetic acid, potassium acid phosphate, cane sugar, and ethyl mercuric bromide. Effects of the series of dust treatments on both greenwood and dormant cuttings have been previously described (13). There were six replicates of the 32 treatments; three replicates were sprayed with wax. The experiment required 1920 cuttings.

The wax emulsion¹ is one designed to reduce transpiration and is recommended for use with conifer transplants. The emulsion, diluted with two volumes of distilled water, was sprayed over the entire cutting with the exception of the basal two centimetres which were covered during application. Groups of cuttings were held for 30 min. before planting to permit the wax to harden.

ARRANGEMENT OF EXPERIMENTS

All experiments were arranged according to the principles of experimental design with treatments replicated and the groups randomized in the propagating frames (28). The data were analysed by the analysis of variance procedure as illustrated by Tables I and II. Data on numbers of cuttings were subjected to the inverse sine transformation prior to analysis (1).

OBSERVATIONS

Cuttings were removed approximately one year after planting and record was taken of the number of cuttings surviving, callused, rooted, bearing new growth, with roots and new growth, and the number and length of roots. The number and length of roots per rooted cutting and the mean root length could be calculated from these data. Approximately four months after the spring plantings, record was made of the number of cuttings surviving and with living new growth.

Results

The results of Experiments 1 and 2, dealing chiefly with nutrient and disinfectant chemicals, are described in detail. The results of the other experiments are summarized to demonstrate the effects of treatment.

EXPERIMENT 1

EFFECTS OF INDOLYLACETIC ACID, CANE SUGAR, AND ETHYL MERCURIC PHOSPHATE

Cane sugar and organic mercury treatment each increased rooting in the absence of the other; in combination there was no beneficial effect.

¹ Dowax, purchased from the Dow Chemical Company, Midland, Michigan, U.S.A.

TABLE I

EXPERIMENT 2. ANALYSIS OF VARIANCE OF RESPONSES OF NORWAY SPRUCE CUTTINGS TREATED WITH TALC DUSTS CONTAINING INDOLYLACETIC ACID, POTASSIUM ACID PHOSPHATE, CANE SUGAR, AND ORGANIC MERCURY AND SPRAYED WITH A WAX EMULSION

Source of variance	Degrees of freedom	Mean square			
		Number of cuttings			
		Rooted	Alive, not rooted	Living, new growth	Dead, new growth
Replicates	2	2362 8***	585 1*	603 0	181 5
Treatments—					
Indolylacetic acid	1	94 9	641 7	619 9	354 8
Organic mercury	1	780 1	5 7	94 9	5 7
Phosphate	3	117 4	754 1**	1320 1***	655 4**
Cane sugar	1	223 2	478 2	24 8	20 7
Interactions—					
Indolylacetic acid × organic mercury	1	141 8	641 7	5 7	86 7
Indolylacetic acid × phosphate	3	201 9	176 4	189 1	28 4
Indolylacetic acid × cane sugar	1	223 2	1 2	1068 8*	131 7
Organic mercury × phosphate	3	63 6	11 7	32 0	229 6
Organic mercury × cane sugar	1	405 4	24 8	379 7	1931 7***
Phosphate × cane sugar	3	253 9	63 4	413 4	461 8*
Indolylacetic acid × organic mercury × phosphate	3	81 6	76 2	603 6*	285 6
Indolylacetic acid × phosphate × cane sugar	3	58 2	62 4	22 2	42 3
Indolylacetic acid × organic mercury × cane sugar	1	5 7	186 0	143 4	391 2
Organic mercury × phosphate × cane sugar	3	173 4	176 8	282 1	128 6
Indolylacetic acid × organic mercury × phosphate × cane sugar	3	123 5	141 2	116 6	351 6
Error (a)	62	213 9	165 5	213 4	158 1
Waxed vs. unwaxed	1	41506 9***	5260 5***	5775 1***	4890 4***
Interactions treatments × wax—					
Indolylacetic acid × wax	1	1068 8*	64 2	2047 5**	2458 2***
Organic mercury × wax	1	121 9	152 3	0 4	0 4
Phosphate × wax	3	92 7	376 6	57 9	245 1
Cane sugar × wax	1	459 4	263 7	756 1*	307 6
Indolylacetic acid × organic mercury × wax	1	13 6	20 7	7 9	34 0
Indolylacetic acid × phosphate × wax	3	89 8	7 7	155 7	29 3
Indolylacetic acid × cane sugar × wax	1	247 7	0 1	423 0	10 5
Organic mercury × phosphate × wax	3	343 9	159 6	12 3	242 8
Organic mercury × cane sugar × wax	1	598 6	263 7	1040 7*	441 1
Phosphate × cane sugar × wax	3	79 2	56 2	333 2	102 2
Indolylacetic acid × organic mercury × phosphate × wax	3	118 3	12 9	226 1	182 1
Indolylacetic acid × phosphate × cane sugar × wax	3	69 0	42 1	163 9	101 1
Indolylacetic acid × organic mercury × cane sugar × wax	1	1 2	1 2	1 2	3 8
Organic mercury × phosphate × cane sugar × wax	3	175 6	249 1	126 8	109 8
Indolylacetic acid × organic mercury × phosphate × cane sugar × wax	3	282 4	176 4	297 1	405 8*
Error (b)	64	177 7	215 8	185 6	129 5

* Exceeds mean square error, 5% level of significance.

** Exceeds mean square error, 1% level of significance.

*** Exceeds mean square error, 0.1% level of significance.

TABLE II

EXPERIMENT 2. ANALYSIS OF VARIANCE OF RESPONSES OF NORWAY SPRUCE CUTTINGS TREATED WITH TALC DUSTS CONTAINING INDOLYLACETIC ACID, POTASSIUM ACID PHOSPHATE, CANE SUGAR, AND ORGANIC MERCURY

Source of variance	Degrees of freedom	Mean square		
		Number of roots per rooted cutting	Length of root per rooted cutting	Mean root length
Replicates	2	0 75	678 5	678 5**
Treatments—				
Indolylacetic acid	1	01	145 0	12 0
Organic mercury	1	18	0 2	135 4
Phosphate	3	96	1176 2	169 7
Cane sugar	1	47	1944 0	9 4
Interactions—				
Indolylacetic acid × organic mercury	1	1 28	181 5	3 4
Indolylacetic acid × phosphate	3	0 72	464 8	68 6
Indolylacetic acid × cane sugar	1	01	5766 0*	495 0
Organic mercury × phosphate	3	19	2423 6	468 9*
Organic mercury × cane sugar	1	00	2147 0	92 0
Phosphate × cane sugar	3	20	316 5	67 4
Indolylacetic acid × organic mercury × phosphate	3	37	1997 1	332 7
Indolylacetic acid × phosphate × cane sugar	3	1 07	5525 7**	419 1*
Indolylacetic acid × organic mercury × cane sugar	1	0 01	1162 0	234 4
Organic mercury × phosphate × cane sugar	3	29	199 2	103 1
Indolylacetic acid × organic mercury × phosphate × cane sugar	3	83	37 6	161 7
Error	62	43	943 6	132 2

* Exceeds mean square error, 5% level of significance.

** Exceeds mean square error, 1% level of significance.

Results for rooting and survival indicated similar interactions between indolylacetic acid and cane sugar treatments, each being beneficial in the absence of the other. In the absence of sugar, indolylacetic acid, on the average, gave 25% rooting as compared to 8% for the controls. Early survival counts indicated beneficial effects from indolylacetic acid and cane sugar treatments on upper but not on lower cuttings. Conversely, the combination of organic mercury and indolylacetic acid increased survival in lower but not upper cuttings.

EXPERIMENT 2

EFFECTS OF POTASSIUM ACID PHOSPHATE, CANE SUGAR, ETHYL MERCURIC BROMIDE, INDOLYLACETIC ACID, AND A WAX SPRAY

In Table I are given the results of statistical treatment of data for the number of cuttings rooted, the number alive but not rooted, and the number with living and dead new growth; the data for the last two items were taken in late summer after the cuttings had been four months in the bed. Similar

results are given in Table II for the numbers and lengths of roots per rooted cutting and the mean root length; these results refer only to the unwaxed cuttings. It is apparent that there were numerous significant primary effects and interactions, and the more important of these are discussed.

Number of Rooted and Surviving Cuttings

Average rooting was 22% for the waxed and 64% for the unwaxed cuttings. Indolylacetic acid treatment gave 25% rooting for waxed cuttings as compared to 18% for the controls but was without effect on the unwaxed. At the end of the experiment 35% of the waxed and 21% of the unwaxed cuttings were alive but not rooted; the corresponding mortality was 44% and 15%. The data in Table III indicate that all concentrations of phosphate tended to reduce the number of living non-rooted cuttings.

Number of Cuttings with New Growth

The early survival count of new growth indicated that 16% of the cuttings had lost the opening bud. Wax treatment resulted in 28% of cuttings with living and 55% with dead new growth. Corresponding percentages for the unwaxed were 15 and 70. The data of Table III indicate that phosphate treatment tended to increase survival of new growth.

TABLE III

EXPERIMENT 2. AVERAGE EFFECTS OF POTASSIUM ACID PHOSPHATE ON NORWAY SPRUCE CUTTINGS

	Potassium acid phosphate in talc, %				Necessary difference, 5% level
	0	0.1	1	10	
Number of cuttings living, not rooted					
Transformed data	34.6	31.2	27.9	25.5	5.3
Per cent	36.7	29.8	25.8	19.6	
Number of cuttings, with living new growth					
Transformed data	19.7	21.4	21.9	31.3	6.0
Per cent	17.3	18.5	19.2	30.0	
Number of cuttings, with dead new growth					
Transformed data	56.1	55.2	54.2	47.9	5.1
Per cent	67.1	66.3	63.3	54.2	

Indolylacetic acid treatment decreased the number of waxed cuttings with living new growth but had no effect on the unwaxed. Cane sugar and organic mercury treatments, on the average, increased the survival of new growth in the absence of wax treatment. Combination of organic mercury with the 10% concentration of phosphate was beneficial in the presence of indolylacetic acid. Both cane sugar and organic mercury treatments tended to increase mortality of new growth, but when in combination there was no injurious effect. Cane sugar and phosphate treatments decreased mortality, but their combination effected no further decrease.

nal examination revealed that only 12% of the cuttings had living 1939 growth. About 4% of the cuttings had 1940 growth, slightly less than half of which came directly from 1938 wood, 1939 growth being absent. Practically all cuttings with 1940 growth were rooted, but a number of cuttings with living 1939 growth were not rooted; this was the more marked with waxed cuttings. Only 23% of the rooted cuttings had living new growth.

Number and Length of Roots per Rooted Cutting

The average number of roots per rooted cutting was 3.1, the average length, 11.1 cm. Data for the interaction effects of indolylacetic acid and cane sugar treatments on the length of root are given in Table IV. Whereas each chemical separately, on the average, tended to increase root length, the combination reduced length. The 10% phosphate concentration increased root length when in combination with either indolylacetic acid or cane sugar, but not with both.

TABLE IV

EXPERIMENT 2. AVERAGE EFFECTS OF INDOLYLACETIC ACID AND CANE SUGAR TREATMENT ON THE LENGTH OF ROOT PER ROOTED CUTTING, CM.

Indolylacetic acid in talc, p.p.m.	Cane sugar in talc, %	
	0	10
0	10.9	11.5
1000	12.2	9.7

Necessary difference, 5% level of significance: 1.8.

Mean Root Length

The mean root length averaged 3.7 cm. The combination of organic mercury and the 10% phosphate concentration resulted in increased length, though the chemicals were somewhat more effective when used separately. The interaction between the 10% phosphate concentration, indolylacetic acid, and cane sugar was similar to that discussed for length of root per rooted cutting.

EFFECTS OF TALC TREATMENT

Talc treatment increased rooting of spring and early summer collections of Norway spruce cuttings propagated in sand. Rooting of cuttings from the lower portions of the tree taken in early May was 23% after talc treatment, as compared to 4% for the controls; upper cuttings failed to show any increase. Early June collections, taken just as new growth was breaking on the cuttings, gave 70% rooting, as compared to 25% for the controls. Summer collections of new growth taken in July and August responded favourably to talc treatment; rooting was increased from 13% for the controls to 40%. Talc treatment effected some increases in root length, particularly with cuttings taken just as new growth was developing. At other seasons of the year, or when

propagation occurred in sand-peat mixture, talc treatment had no effect on rooting, though in some instances there were increases in the length of root per rooted cutting. Delay of 24 hr. in planting after preparation and treatment of cuttings was followed by beneficial effects from talc treatment.

EFFECTS OF INDOLYLACETIC ACID TREATMENT

Indolylacetic acid treatment in some cases suggested beneficial effects in the number rooted and the number and lengths of roots when the cuttings were taken just prior to the emergence of new growth and propagated in sand. It also increased rooting of waxed cuttings (Experiment 2). In sand-peat mixtures and in the other experiments in sand, indolylacetic acid was not beneficial. Sometimes it was damaging, almost always when used in concentrations of 8000 and 10,000 p.p.m.

EFFECTS OF WAXING

The application of wax to Norway and white spruce cuttings had no effect on rooting or mortality during dormancy. It was injurious to cuttings with an emerging shoot, and to greenwood cuttings. Injurious effects of wax treatment on eastern white cedar cuttings were restricted to untreated and talc treated cuttings. In combination with 5, 100, and 1000 p.p.m. of indolylacetic acid, waxing had no adverse effects on mortality.

Discussion

Phytohormone, nutrient, and disinfectant chemicals, applied as dusts, have been shown to affect the responses of Norway spruce cuttings. The beneficial effects have been of small magnitude, and the same chemical may be beneficial, without effect, or damaging, depending on conditions. Hitchcock and Zimmerman (19) have emphasized that many conditions, e.g., age and relative activity of the shoot, time of year, and method of applying the substance, affect the response of cuttings to chemicals. Komissarov (25) and Kirkpatrick (23) have both pointed out that high temperatures are necessary for root-inducing substances to be effective. This is contrary to our experience in which cuttings treated in November and left outdoors in subzero temperatures over winter responded very favourably to treatment (17) and cuttings treated in summer did not respond at all. This is not to be taken as a contradiction but merely an illustration of the importance of other factors relating to the environment and the nature of the cuttings themselves in obtaining a response to treatment.

In the example just mentioned (17) the response to treatment with indolylacetic acid was modified by the medium in which the cuttings were grown. Experiments herein reported show that the part of the tree from which the cuttings are taken also affects their response to treatment.

Although indolylacetic acid is undoubtedly advantageous under certain conditions, its use in the propagation of Norway spruce does not seem to be warranted, at least until a number of other factors become better understood.

he beneficial results from talc treatment are in agreement with earlier findings dealing with conifers and other plants (11, 12, 14, 19, 21, 27, 30). The favourable response to talc when planting had been delayed, and when the cuttings were put in a relatively dry medium, sand, corroborates the suggestion of Hitchcock and Zimmerman (19) that talc affects the water relations of the cuttings. Damage due to talc treatment was observed in some experiments and has been previously reported (14). This variability is no doubt another illustration of conditions affecting the response to treatment.

The beneficial effect of nutrient on the rooting of Norway spruce cuttings has been reported by the authors (7, 14); a complete nutrient, in solution, was applied at weekly intervals. Since that method is tedious, in the experiments reported here certain nutrient chemicals were mixed with a carrier dust and applied to the cuttings in a single treatment. Potassium phosphate was chosen since phosphates are usually associated with root growth; potassium is an absolutely essential element, and all meristematic tissues are rich with it (26). However, the effect of this chemical seemed to be concerned with the development of buds rather than the initiation of roots. Likewise, when cuttings of Japanese yew (*Taxus cuspidata* Sieb. and Zucc.) were treated with a dust containing complete nutrient the main effects were on the buds (16). An increase in new growth was noticed in the original experiment with nutrient watering (7).

Both ethyl mercuric phosphate and ethyl mercuric bromide, as disinfectant chemicals, have been used but no essential differences in their effects have been noted. The purpose of these chemicals was to protect the bases of the cuttings from fungi (8). However, it turned out that the effects were concerned with new growth. In previous work (17) mercury influenced rooting favourably to a small extent. In other experiments (10, 14) mercury reduced rooting.

Sugar is thought to supply the cutting with energy during the period prior to root formation. Its effects were not great in Experiment 2, and in previous work (10, 14, 17) the results were variable.

The results of treatments with various chemicals do not indicate any essential differences between the effects of phytohormone chemicals and the others. This would seem to corroborate the opinion recently expressed by Swingle (31) that in dealing with root-forming substances the effects of materials foreign to the plant are being studied.

The detrimental effects of waxing are in agreement with a recent report on the effect of the same wax emulsion on white pine cuttings (2).

Success in vegetative propagation of conifers appears to relate to conditions of propagation, type of cuttings, and seasonal variations of the parent plant, rather than to any chemical treatments. Elucidation of the physico-chemical bases for the association of these factors must await further experimental study.

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AGRICULTURAL METEOROLOGY: SEASONAL INCIDENCE OF RAINGLESS AND RAINY PERIODS AT WINNIPEG, SWIFT CURRENT, AND EDMONTON¹

By J. W. HOPKINS²

Abstract

The frequency of sequences of consecutive days without rain and with rain at each of the above-named meteorological stations has been determined from their records of daily precipitation for the months April to September of the years 1916 to 1937. It is inferred from these frequencies that rainy or rainless days do not in general occur entirely at random, but that the same kind of weather tends to persist over successive days. The statistics have also been used to estimate the expectation of rainless periods. At all three stations, this is least in mid-summer (June and July) and greatest in spring and autumn.

Introduction

In a recent Technical Bulletin of the United States Department of Agriculture, Blumenstock (1) points out that lengthy rainless intervals are an important factor in the initiation of soil erosion by wind action, and that it is therefore desirable to ascertain how periods without precipitation vary in length from place to place and from season to season of the year, in order to arrive at drought-expectancy figures from which this particular climatic risk may be evaluated. The writer has extracted from the published records of the Meteorological Service of Canada (3) statistics of the daily occurrence of rains during the summer period April 1 to September 30 of the 22 years, 1916-1937, at three representative meteorological stations in the Prairie Provinces of Canada; these are presented and discussed in the following sections of this paper. The three stations selected for this purpose were Winnipeg, Manitoba (lat. 49° 53' N., long. 97° 7' W., alt. 760 ft.), Swift Current, Saskatchewan (50° 20' N., 107° 45' W., 2440 ft.), and Edmonton, Alberta (53° 33' N., 113° 30' W., 2158 ft.)

Data

Table I shows the total number of days, during the six months of each year considered, on which no measurable amount of precipitation (i.e., less than 0.01 in. of rain) was recorded. As has been noted elsewhere (6) the number of rainless days is on the average least during midsummer, but the seasonal trend in this respect is less than proportional to the marked trend in the actual amounts of rain received.

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TABLE I
NUMBER OF RAINLESS DAYS PER MONTH, 1916-1937, AT WINNIPEG, SWIFT CURRENT,
AND EDMONTON

Year	Apr.	May	June	July	Aug.	Sept.
Winnipeg						
1916	25	18	18	25	22	20
1917	25	30	18	21	20	22
1918	24	24	18	20	18	20
1919	25	24	23	24	21	18
1920	23	25	20	25	22	19
1921	22	22	20	21	21	16
1922	22	19	20	22	24	20
1923	24	24	25	15	23	20
1924	14	26	21	22	20	21
1925	20	24	11	22	16	24
1926	25	21	19	26	19	16
1927	19	18	16	20	22	19
1928	21	26	17	17	17	24
1929	25	23	20	23	26	25
1930	24	15	19	22	22	23
1931	25	24	22	15	23	18
1932	21	27	19	19	23	23
1933	22	27	22	17	21	20
1934	22	22	16	21	22	15
1935	19	25	16	22	19	16
1936	21	23	21	24	29	25
1937	17	21	19	22	20	18
Mean	22 0	23 1	17 2	21 2	21 4	20 1
Standard deviation	2 9	3 5	2 9	3 0	2 9	3 0
χ^2	30 952	42.749**	24.519	28 440	26.062	28 906
Swift Current						
1916	25	15	15	20	22	22
1917	19	29	21	25	22	23
1918	24	24	23	21	20	27
1919	23	25	26	27	23	23
1920	20	22	17	24	26	24
1921	24	20	20	19	23	21
1922	22	18	18	22	22	25
1923	22	23	14	14	23	29
1924	24	25	14	21	17	24
1925	17	26	20	23	22	19
1926	24	19	17	24	20	22
1927	18	11	20	16	25	21
1928	23	26	12	19	21	28
1929	21	24	23	25	27	21
1930	21	25	21	22	22	22
1931	24	26	20	23	25	22
1932	19	24	16	21	20	24
1933	22	24	20	27	22	21
1934	26	19	11	23	25	17
1935	18	23	14	19	25	28
1936	24	24	16	25	26	26
1937	25	21	24	22	24	22
Mean	22 0	22.4	18.3	21.9	22.8	23 2
Standard deviation	2.6	4.1	4.0	3.2	2.4	3.0
χ^2	24 110	56.575**	45.842**	34.520*	20.470	36.202*

TABLE I—*Concluded*
 NUMBER OF RAINLESS DAYS PER MONTH, 1916–1937, AT WINNIPEG, SWIFT CURRENT,
 AND EDMONTON—*Concluded*

Year	Apr.	May	June	July	Aug.	Sept.
Edmonton						
1916	24	16	21	18	20	19
1917	21	21	18	18	15	23
1918	23	17	14	18	9	25
1919	22	23	22	16	20	18
1920	18	18	16	23	24	24
1921	25	21	17	16	19	23
1922	24	24	19	18	17	25
1923	26	21	16	15	15	24
1924	20	25	17	14	15	25
1925	22	24	17	21	16	20
1926	25	22	18	26	16	15
1927	27	20	17	14	23	15
1928	15	26	12	18	15	27
1929	16	22	21	18	18	24
1930	23	21	10	22	19	17
1931	28	22	15	15	18	14
1932	18	26	19	16	25	23
1933	20	19	14	11	23	16
1934	25	20	11	20	18	12
1935	25	22	11	18	14	22
1936	22	20	15	19	19	21
1937	21	21	16	11	16	21
Mean	22 3	21 4	16 2	17 5	17 9	20 6
Standard deviation	3 4	2 6	3 3	3 6	3 7	4 2
χ^2	43 289**	21 939	30 492	35 890*	38 586*	58 429**

* Exceeds 5% point (32 671).

** Exceeds 1% point (38 932).

Tables II, III, and IV give the frequency of occurrence, at each station, of rainless periods of varying length, following each rainy day, during the 22 years, and Table V gives similar information in respect of sequences of rainy days following each dry day. It requires to be noted that whereas the frequencies listed in Table I are based strictly on calendar months, some elasticity of definition had to be introduced in order to avoid the arbitrary truncation in Tables II to V of natural periods, either wet or dry, overlapping from one month into the next. Accordingly, if a natural sequence of 15 rainless days, for example, began on June 20, so that 11 of the days involved fell in June and four in July, the entire sequence of 15 would be credited to the month of June. On the other hand, had the date of commencement been June 25, so that the major part of the sequence actually occurred in July, the entire sequence would have been credited to the latter month. It may be anticipated that additions to and subtractions from the records for each month occasioned in this way will approximately balance.

TABLE II

FREQUENCY OF OCCURRENCE OF RAINLESS PERIODS OF SPECIFIED LENGTH, WINNIPEG, 1916-1937

Rainless period days	Apr		May		June		July		Aug		Sept	
	Ob- served	Random exp	Ob- served	Random exp	Ob- served	Random exp	Ob- served	Random exp	Ob- served	Random exp	Ob- served	Random exp
0	61	44.3	68	48.0	96	103.0	72	70.0	82	64.7	93	73.3
1	28	32.5	33	35.7	58	59.2	48	47.7	34	44.6	40	49.1
2	18	23.9	23	26.6	36	34.0	23	32.5	24	30.7	26	32.9
3	9	17.6	13	19.8	20	19.5	22	22.2	25	21.2	16	22.0
4	11	12.9	12	14.8	9	11.2	24	15.1	11	14.6	18	14.8
5	8	9.5	8	11.0	5	6.4	13	10.3	12	10.0	10	9.9
6	7	7.0	8	8.2	6	3.7	5	7.0	6	6.9	6	6.6
7	4	5.1	4	6.1	0	2.1	4	4.8	0	4.8	4	4.4
8	4	3.8	1	4.6	3	1.2	3	3.3	3	3.3	0	3.0
9	5	2.8	2	3.4	5	0.7	3	2.2	3	2.3	2	2.0
10	1	2.0	3	2.5	3	0.4	1	1.5	2	1.6	1	1.3
11	2	1.5	3	1.9	0	0.2	1	1.0	0	1.1	1	0.9
12	3	1.1	3	1.4	0	0.1	0	0.7	0	0.7	0	0.6
13	2	0.8	1	1.0	0	0.1	1	0.5	0	0.5	1	0.4
14	1	0.6	1	0.8	1	0.04			2	0.4	0	0.3
15	1	0.4	1	0.6					1	0.2	0	0.2
16	0	0.3	3	0.4					1	0.2	1	0.1
17	0	0.2	0	0.3					0	0.1	0	0.1
18	0	0.2	0	0.2					2	0.1	1	0.05
19	2	0.1	0	0.2							1	0.04
20			0	0.1							0	0.02
21			0	0.1							0	0.02
22			0	0.1							0	0.01
23			0	0.05							0	0.01
24			0	0.04							0	0.01
25			0	0.03							1	0.003
26			0	0.02								
27			0	0.02								
28			0	0.01								
29			0	0.01								
30			0	0.01								
31			0	0.01								
32			1	0.004								
χ^2	17 403*		20 181*		11 848		9 513		15 910*		13 754	

* Exceeds 5% point.

Length and Frequency of Recorded Sequences

In the bulletin to which reference has already been made, Blumenstock (1) is of the opinion that there is a linear relation between the length of a rainless period and the logarithm of the frequency of its occurrence. It is of interest to note that, for this to be so, the probability of rain occurring on any particular day must be statistically independent of the weather of the preceding day or days. In this event, let p be the probability of a day being rainy, and $q (= 1 - p)$ the complementary probability of its being rainless. Then if rain occurs on any specified day, the probability that the next day will also be rainy is p . On the other hand the probability that the next day will be fine, but that rain will occur again on the second next day, will be qp ; and in

TABLE III

FREQUENCY OF OCCURRENCE OF RAINLESS PERIODS OF SPECIFIED LENGTH, SWIFT CURRENT, 1916-1937

Rainless period, days	Apr.		May		June		July		Aug.		Sept.	
	Observed	Random exp.	Observed	Random exp.	Observed	Random exp.	Observed	Random exp.	Observed	Random exp.	Observed	Random exp.
0	79	46 1	96	55 1	133	99 3	77	58 7	72	46 7	48	33 2
1	21	33 9	24	39 9	35	60 5	29	41 5	23	34 4	27	25 7
2	13	24 9	17	28 8	31	36 8	27	29 3	10	25 3	13	19 9
3	7	18 3	19	20 8	15	22 4	11	20 7	20	18 6	13	15 4
4	15	13 4	5	15 1	10	13 7	15	14 6	8	13 7	5	11 9
5	9	9 9	7	10 9	10	8 3	9	10 3	14	10 1	6	9 2
6	4	7 3	6	7 9	6	5 1	8	7 3	8	7 4	6	7 2
7	3	5 3	7	5 7	3	3 1	13	5 2	7	5 5	3	5 5
8	5	3 9	3	4 1	7	1 9	5	3 6	2	4 0	5	4 3
9	2	2 9	3	3 0	1	1 2	3	2 6	3	3 0	1	3 3
10	1	2 1	1	2 2	2	0 7	1	1 8	2	2 2	2	2 6
11	3	1 6	1	1 6	0	0 4	0	1 3	2	1 6	5	2 0
12	2	1 1	0	1 1	0	0 3	0	0 9	0	1 2	2	1 5
13	5	0 8	3	0 8	0	0 2	0	0 6	3	0 9	0	1 2
14	1	0 6	1	0 6	0	0 1	0	0 4	0	0 6	1	0 9
15	4	0 4	0	0 4	0	0 1	0	0 3	0	0 5	1	0 7
16			2	0 3	0	0 04	0	0 2	1	0 4	2	0 6
17			1	0 2	1	0 02	0	0 2	0	0 3	1	0 4
18			1	0 2			0	0 1	0	0 2	0	0 3
19			1	0 1			0	0 1	1	0 1	2	0 3
20			0	0 1			0	0 1	0	0 1	0	0 2
21			0	0 1			2	0 04	0	0 1	1	0 2
22			1	0 04					0	0 1	0	0 1
23									0	0 04	1	0 1
24									1	0 03	0	0 1
25											0	0 1
26											0	0 04
27											0	0 03
28											0	0 03
29											1	0 02
30											0	0 02
31											0	0 01
32											0	0 01
33											0	0 01
34											0	0 01
35											1	0 004
χ^2	63 076**		53 106**		31 428**		28 191**		32 066**		23 467**	

** Exceeds 1% point.

general the probability of a sequence of 0, 1, 2, . . . n rainless days, terminated by rain on the 1st, 2nd, 3rd, . . . $n + 1$ th days will be given by successive terms of the geometric series

$$p, qp, q^2p, \dots, q^n p$$

(2, 4) from which the logarithm of the probability of a sequence of exactly n dry days is

$$\log p + n \log q,$$

a linear function of n as postulated.

A further requirement for linearity in the case of observations extending over a period of years is that p must remain constant throughout. If this

TABLE IV

FREQUENCY OF OCCURRENCE OF RAINLESS PERIODS OF SPECIFIED LENGTH, EDMONTON, 1916-1937

Rainless period, days	Apr.		May		June		July		Aug.		Sept.	
	Ob-served	Random exp.	Ob-served	Random exp.	Ob-served	Random exp.	Ob-served	Random exp.	Ob-served	Random exp.	Ob-served	Random exp.
0	76	44.8	105	68.4	166	137.7	163	133.3	139	117.8	97	61.8
1	25	33.3	29	47.2	50	74.3	56	75.2	54	68.1	20	42.4
2	15	24.7	25	32.6	36	40.1	29	42.5	26	39.3	25	29.1
3	13	18.3	14	22.5	15	21.6	18	24.0	23	22.7	11	20.0
4	7	13.6	9	15.6	15	11.7	12	13.5	13	13.1	9	13.7
5	6	10.1	11	10.7	7	6.3	11	7.6	6	7.6	12	9.4
6	9	7.5	6	7.4	5	3.4	3	4.3	8	4.4	5	6.5
7	5	5.6	6	5.1	0	1.8	8	2.4	6	2.5	2	4.4
8	3	4.1	3	3.5	1	1.0	2	1.4	2	1.5	4	3.0
9	2	3.1	3	2.4	1	0.5	3	0.8	0	0.8	2	2.1
10	1	2.3	3	1.7	1	0.3	0	0.4	2	0.5	3	1.4
11	4	1.7	1	1.2	1	0.2	1	0.2			0	1.0
12	1	1.3	1	0.8	0	0.1					1	0.7
13	3	0.9	0	0.6	1	0.05					2	0.5
14	0	0.7	2	0.4							1	0.3
15	1	0.5	0	0.3							0	0.2
16	0	0.4	0	0.2							1	0.2
17	1	0.3	0	0.1							0	0.1
18	1	0.2	2	0.1							1	0.1
19	0	0.2	1	0.1							0	0.05
20	0	0.1									0	0.04
21	0	0.1									0	0.02
22	0	0.1									0	0.02
23	0	0.05									0	0.01
24	0	0.04									0	0.01
25	1	0.03									1	0.01
χ^2	37 223**		38 493**		18 163**		23 459**		18 679**		43 515**	

** Exceeds 1% point.

is not so, and p fluctuates from year to year, the relative frequency of sequences of 0, 1, 2, 3, etc. rainless days during the period as a whole will constitute a series which is compounded of a number of separate geometric series, but which is not itself geometric.

A priori, it might perhaps be considered unlikely that the meteorological conditions prevailing in the Prairie Provinces would satisfy either of the foregoing requirements. A major part of the precipitation in this area is associated with the passage of low pressure areas of such an extent that the weather of successive days might be affected by the same "low", and hence could hardly be regarded as statistically independent. Furthermore, there is generally some secular variation in the rainfall recorded at any point over a period of years, alternating periods of above- and below-average precipitation, which might be expected to affect the value of p , tending to occur at irregular intervals (6).

Judging from the observations recorded in Tables I to IV, it would seem that both of these factors are in practice operative. Thus in order to test

TABLE V

FREQUENCY OF OCCURRENCE OF SEQUENCES OF DAYS WITH RAIN, WINNIPEG, SWIFT CURRENT, AND EDMONTON, 1916-1937

Number of consecutive days with rain	Apr.		May		June		July		Aug.		Sept.	
	Observed	Random exp.	Observed	Random exp.	Observed	Random exp.	Observed	Random exp.	Observed	Random exp.	Observed	Random exp.
Winnipeg:												
0	376	355.6	407	388.1	254	232.6	310	313.0	332	317.0	327	307.4
1	62	94.3	73	99.0	97	99.0	97	99.6	73	98.6	73	101.5
2	33	25.0	24	25.3	30	42.2	41	31.7	33	30.6	40	33.5
3	6	6.6	12	6.4	11	18.0	5	10.0	18	9.5	12	11.1
4	6	1.8	4	1.6	9	7.6	3	3.2	3	3.0	2	3.7
5	0	0.5	2	0.4	3	3.2	3	1.0	1	0.9	3	1.2
6	1	0.1			1	1.4					1	0.4
7											1	0.1
Swift Current:												
0	386	357.1	385	353.5	274	241.2	356	335.0	378	360.0	461	428.1
1	55	94.7	53	98.0	55	94.3	66	98.2	59	95.0	58	96.7
2	28	25.1	28	27.1	35	36.9	33	28.8	39	25.1	25	21.8
3	9	6.7	15	7.5	16	14.4	14	8.4	11	6.6	4	4.9
4	2	1.8	2	2.1	8	5.6	5	2.5	1	1.8	5	1.1
5	5	0.5	1	0.6	4	2.2			0	0.5		
6	0	0.1	3	0.2	1	0.9			0	0.1		
7	0	0.03	1	0.04	1	0.3			0	0.03		
8	1	0.01	1	0.01	1	0.1			0	0.01		
9					1	0.05			1	0.002		
Edmonton:												
0	365	341.5	360	328.0	210	189.9	258	225.2	245	221.3	322	291.7
1	49	88.0	62	101.5	60	87.4	65	98.1	62	93.4	43	91.5
2	31	22.7	27	31.4	40	40.3	33	42.7	40	39.5	36	28.7
3	6	5.8	10	9.7	18	18.6	22	18.6	19	16.7	18	9.0
4	5	1.5	11	3.0	11	8.6	12	8.1	11	7.0	3	2.8
5	2	0.4	1	0.9	9	3.9	4	3.5	3	3.0	1	0.9
6	2	0.1	3	0.3	3	1.8	1	1.5	2	1.2	1	0.3
7			1	0.1	1	0.8	2	0.7	1	0.5	0	0.1
8							2	0.3			1	0.03

the constancy of q , and hence also of $p = 1 - q$, the index of dispersion χ^2 appropriate to a binomial distribution was calculated (following the procedure of Fisher (5)) from the annual variation in the number of rainless days recorded in each month at the three stations; it is shown at the foot of the corresponding column in Table I. Of the aggregate 18 values of χ^2 computed in this way, nine exceed the 5% and five exceed the 1% point, a circumstance that is hardly compatible with the assumption that for any specified month and station, e.g., April at Edmonton, p and q are in fact the same from year to year.

The frequency distributions of sequences of rainless days given in Tables II to IV are also for the most part inconsistent with the expected relative frequencies (listed in the columns headed "random expectation") calculated from the

geometric series on the assumption, as indicated above, that for any specified month and station, the average values of p and q obtained from Table I are applicable to every day covered by the period of record. It is very evident that at both Swift Current (Table III) and Edmonton (Table IV) there was, in all the six months considered, on the one hand an excess of occasions on which a rainy day was immediately followed by another rainy day (shown as sequences of zero dry days) and of dry sequences of six or more days, and on the other a deficiency of rainless periods of one to three days' duration. The statistical significance of these deviations of observation from hypothesis was tested by the calculation of χ^2 in the usual way (5), the terminal frequencies in each case being grouped so as to provide a minimum expectation of five. All 12 of the resulting values of χ^2 for these two stations are in excess of the 1% point.

Similar tendencies are to be noted in the records for Winnipeg (Table II), but here the deviation of the observed frequencies from the random expectation is less marked, and only three of the six values of χ^2 are individually statistically significant. An alternative test, due to Cochran (4), which does not take into consideration the number of "zero sequences" but which involves no grouping of the tail frequencies and consequent loss of information as in the χ^2 test was accordingly applied to these series. It was thus found that the occurrence of lengthy rainless periods in June at this station was also significantly in excess of the random expectation.

The observations as a whole are clearly inconsistent with the purely random occurrence of either rainless or rainy spells (the two being of necessity complementary), but are indicative of a tendency of the prevailing weather to persist over several days, especially at the two more westerly stations. Figs. 1 and 2 illustrate the effect of this on the frequency distribution of rainless and rainy sequences. The deviation from linearity in the relation between length of period and logarithmic frequency is apparent although, owing to the rarity of the lengthier sequences, the tails of the distributions are determined with uncertainty. These accordingly did not seem to merit any more extensive treatment than graduation by the second-degree curves shown, which were fitted by the method of Least Squares.

A readily recognizable climatic characteristic arises from the proportion of occasions on which there is no change in the weather from one day to the next, i.e., on which a rainy day is followed by another rainy day, or a fine day by a second fine day. This proportion may be computed from Tables II to V. Table II, for example, comprises a total of 1247 days with rain, constituting the starting points defining the sequences of dry days recorded at Winnipeg. Of these, 472, giving rise to the tabulated "zero" sequences, were succeeded by another rainy day. Similarly Table V comprises a total of 2788 rainless days at Winnipeg, providing the reference points for the observed sequences

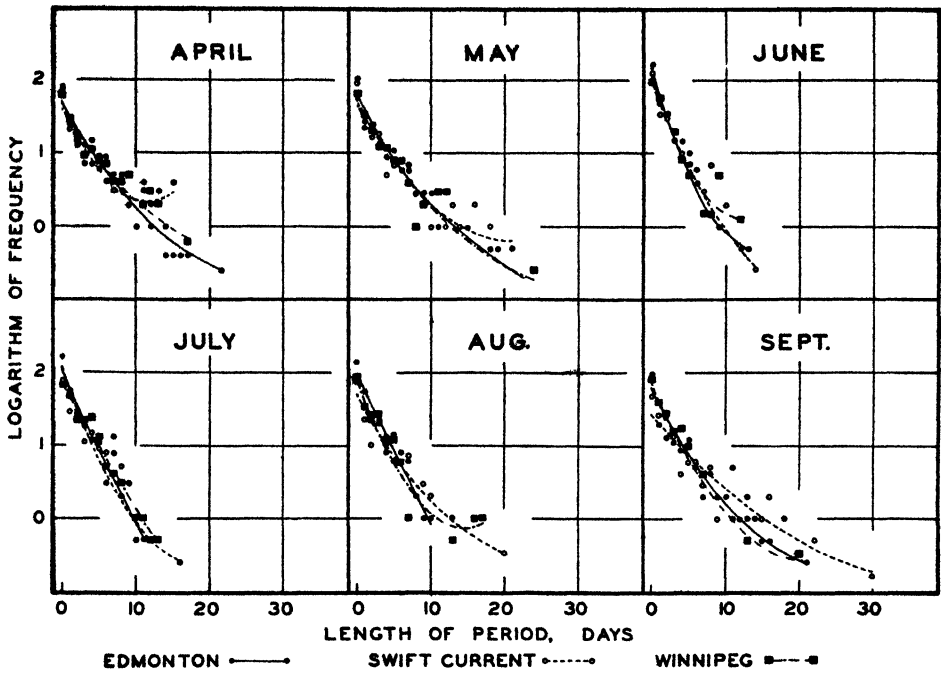


FIG. 1. Logarithmic frequency (1916-1937), by months, of rainless periods of specified length at Winnipeg, Swift Current, and Edmonton.

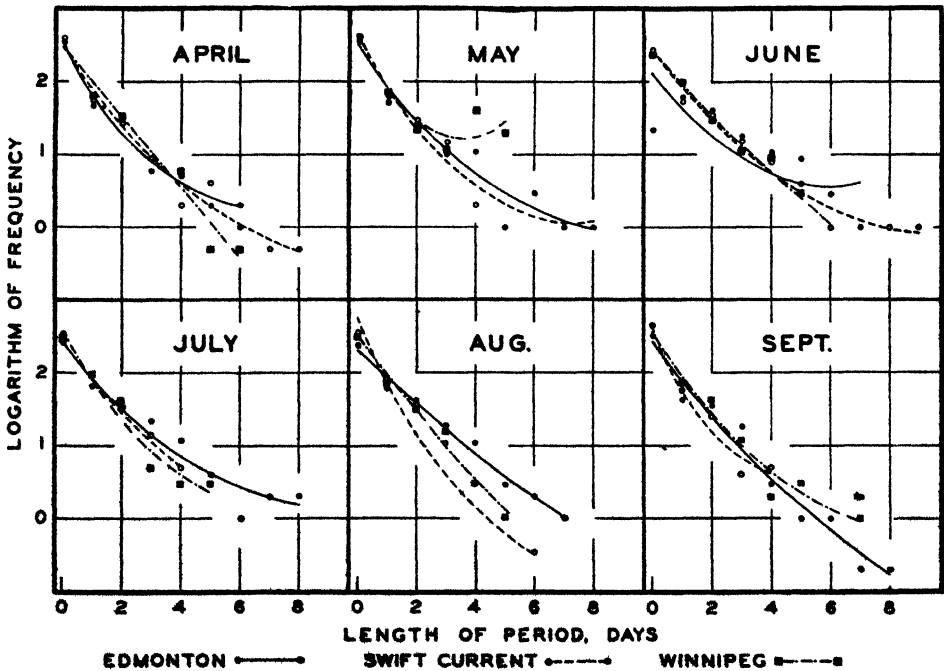


FIG. 2. Logarithmic frequency (1916-1937), by months, of rainy periods of specified length at Winnipeg, Swift Current, and Edmonton.

of rainy days, of which 2006 were followed by another rainless day. Of the total of 4035 days considered therefore, 2478 or 61% were followed by another rainy or rainless day as the case might be. The corresponding percentages for Swift Current and Edmonton are 68 and 63, respectively.

Expectation of Rainless and Rainy Periods

Tables II to IV, each summarizing the experience of 22 years, may be used to make estimates of drought expectancy; the results are shown in Table VI. This last table indicates the length of rainless periods that must be expected on the basis of the (ungraduated) past experience cited to be attained or exceeded once per annum, once in five years, and once in 10 years during the specified month at each station. As the 10-year points are of necessity deduced from only two observed occurrences, they must be regarded as rough

TABLE VI

LENGTH OF RAINLESS PERIODS HAVING SPECIFIED EXPECTED FREQUENCIES

Station	Expected frequency	Length of period, days					
		Apr.	May	June	July	Aug.	Sept.
Winnipeg	Once per year	7	7	5	5	5	5
	Once in 5 years	13	14	9	9	14	11
	Once in 10 years	19	16	10	10	16	18
Swift Current	Once per year	8	7	5	7	7	8
	Once in 5 years	13	16	8	9	13	19
	Once in 10 years	15	18	10	10	16	23
Edmonton	Once per year	7	6	4	5	5	6
	Once in 5 years	13	12	6	8	7	13
	Once in 10 years	17	18	10	9	8	16

TABLE VII

EXPECTED FREQUENCY OF OCCURRENCE OF RAINY PERIODS OF SPECIFIED LENGTH

Station	Length of period, days	Expected frequency of occurrence, once in:					
		Apr.	May	June	July	Aug.	Sept.
Winnipeg	4 or more	3.1 yr.	3.7 yr.	1.7 yr.	3.7 yr.	5.5 yr.	3.1 yr.
	5 or more	22	11	5.5	7.3	22	4.4
	6 or more	22	> 22	22	> 22	> 22	11
Swift Current	4 or more	2.8	2.8	1.4	4.4	11	4.4
	5 or more	4.4	3.7	2.8	> 22	22	> 22
	6 or more	22	4.4	5.5	> 22	22	> 22
Edmonton	4 or more	2.4	1.4	0.9	1.0	1.3	3.7
	5 or more	5.5	4.4	1.7	2.4	3.7	7.3
	6 or more	11	5.5	5.5	4.4	7.3	11



approximations only. On this basis the expectation of rainless periods is least in midsummer (June and July) and greatest in spring and autumn. At Swift Current, for example, it may be anticipated that there will be at least five consecutive June days without rain each year; that eight or more consecutive rainless days during this month may be expected on the average once in five years; and 10 or more once in 10 years on the average. For the month of September on the other hand the corresponding minimum expectations at this station are 8, 19, and 23 days, respectively.

Unduly prolonged rainy periods may also be agriculturally disadvantageous, not only by hindering field work but also in some cases by facilitating or aggravating water erosion of soil (7). It is of course true that under prairie weather conditions the maximum duration of rainy periods recorded is much less than that of rainless ones. Nevertheless, the average frequencies deduced from Table V and recorded in Table VII indicate an appreciable incidence of sequences of as many as five or more consecutive days with measurable amounts of rain, particularly at Swift Current and Edmonton.

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

XII. SUMMARY OF CORRELATIONS BETWEEN 18 MAJOR BARLEY, MALT, AND MALTING PROPERTIES¹

BY J. A. ANDERSON², H. R. SALLANS³, AND W. O. S. MEREDITH⁴

Abstract

A summary is presented of correlation studies based on data, for 11 barley properties and 7 malt properties, obtained by analysis of samples of 12 varieties of barley (and of the malts made from them) grown at 12 experimental stations in Canada. Intra- and intervarietal associations between pairs of properties were studied separately using means over all varieties for each station, and means over all stations for each variety. Simple correlation coefficients for all possible pairs of properties are reported. In addition, intravarietal partial correlation coefficients, independent of total nitrogen, and intervarietal partial correlation coefficients, independent of salt-soluble nitrogen, are also given.

The main purpose of the paper is to put on record, for ready reference, tables of statistics that may be useful to other investigators who are interested in the associations that exist between barley and malt properties, and the light that these throw on the nature of malting quality in barley.

Previous papers in this series (1, 2, 7-9, 16, 17, 19-22) presented the results of studies of the effects of variety and environment on a relatively large number of barley and malt properties. The data furnished conclusive proof that both factors have a significant effect on all the properties measured. It was also possible to examine, by means of correlation studies, certain of the associations that exist between various barley and malt properties, and thus to show that the inter- and intravarietal associations differ materially for many of the pairs of properties examined. It appears that these studies throw some light on the nature of malting quality in barley, a problem that is by no means solved and that will probably continue to attract the attention of malting chemists for many years. Accordingly it seemed useful to put on record in the present paper a brief summary of the results of the correlation studies made during the course of the investigations. A number of these have already been reported. The list is now completed and consists of inter- and intravarietal correlation coefficients for all possible pairs obtainable from 11 barley properties and 7 malt properties. To these have been added the corresponding intervarietal partial correlation coefficients, independent of salt-

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soluble barley nitrogen and the intravarietal partial correlation coefficients independent of total barley nitrogen.

The discussion of these correlation coefficients is not extensive. The main purpose is to put on record, for ready reference, tables of statistics that may be useful to other investigators interested in the same field of research.

Correlation Coefficients

In considering the correlation coefficients in the following sections, the reader should bear in mind that all determinations were made on the same set of 144 barleys and their corresponding malts and that these samples represented 12 varieties of barley grown at 12 widely separated experimental stations (1). The barleys were pure strain varieties grown on replicated randomized plots and the maltings were made in duplicate under carefully controlled and standardized malting conditions (1). Although the number of stations and varieties appears somewhat limited, the wide range in barley types (five, rough-awned six-rowed; four, smooth-awned six-rowed; and three, rough-awned two-rowed, varieties) and the range of 1.5 to 2.8% in the mean nitrogen content for stations suggests that the investigations covered a sufficiently wide range to yield useful estimates of the correlation coefficients.

It might be assumed that the relations reported would apply only under malting conditions that approximate very closely those employed for these investigations. However, studies on the effects of different malting treatments on malt quality (3-6, 18) indicate that when the same set of barleys is malted under quite widely varying conditions the varieties and stations are placed in essentially the same relative order within each treatment. Thus if a different system of malting had been employed in the investigations, it seems altogether probable that correlation coefficients thus obtained would not have differed markedly from those reported. Consequently, it appears that the present study serves to indicate relations that are inherent in barleys and in the malts produced from them by any reasonable and uniform malting procedure.

Obviously the results obtained in the present investigations could not be duplicated with pairs of barley and malt samples selected at random from commercial malt houses. With such a series of samples it would be impossible to separate the effects of variety (i.e., genetic constitution) from the effects of environment on the various barley and malt properties, nor would it be possible to study separately the intravarietal (i.e., interstation) and the intervariational associations between pairs of properties. Moreover, the investigation would be further complicated by such differences as existed between the malting methods used in different malt houses.

Since the intra- and intervariational associations between barley and malt properties differ widely, these are discussed separately in the following subsections.

TABLE I

SIMPLE INTRAVARIETAL CORRELATION COEFFICIENTS AND CORRESPONDING PARTIAL CORRELATION COEFFICIENTS, INDEPENDENT OF TOTAL NITROGEN, FOR BARLEY AND MALT PROPERTIES

Simple correlation coefficient								
B. Total nitrogen, %	.926**	.985**	.851**	-.519	-.953**	.173	-.908**	.924**
—	B. Glutelin nitrogen, %	.878**	.694*	-.527	-.890**	.278	-.857**	.807**
—	-.525	B. Hordein nitrogen, %	.793**	-.447	-.939**	.208	-.897**	.875**
—	-.476	-.496	B. Salt-soluble nitrogen, %	-.579*	-.801**	-.099	-.740**	.966**
—	-.142	.435	-.307	B. 1000-kernel weight	.688*	-.294	.688*	-.552
—	-.062	-.004	.065	.746**	B. Starch, %	-.386	.961**	-.859**
—	.315	.218	-.477	-.242	-.742**	B. Insoluble residue, %	-.420	-.631*
—	-.102	-.036	.149	.605*	.752**	-.637*	B. Extract, %	-.769**
—	.336	-.535	.892**	-.221	.190	.256	.439	B. Wort nitrogen, %
—	-.021	.132	-.108	.623*	.249	.295	.277	-.107
—	-.079	.094	-.017	.652*	.797**	-.738**	.730*	.233
—	.045	.373	.378	.304	.218	-.162	.158	.247
—	.253	-.150	-.118	-.466	-.587	.531	-.203	-.138
—	.022	-.319	.305	.416	.625*	-.569	.854**	.475
—	-.062	.469	-.423	.276	-.221	.591	.022	-.386
—	-.006	.297	-.545	-.022	-.428	.670*	-.643*	-.692*
—	.300	-.010	-.296	-.446	-.420	.458	-.762**	-.566
—	-.078	.238	-.168	-.572	-.817**	.677*	-.674*	-.397

Partial correlation coefficient, independent of total nitrogen

** denotes that the 1% level and * that the 5% level of significance has been attained.

TABLE I

SIMPLE INTRAVARIETAL CORRELATION COEFFICIENTS AND CORRESPONDING PARTIAL CORRELATION COEFFICIENTS, INDEPENDENT OF TOTAL NITROGEN, FOR BARLEY AND MALT PROPERTIES

Simple correlation coefficient								
.976**	-.637*	.694*	.764**	-.957**	.962**	.854**	-.269	.632*
.902**	-.613*	.655*	.769**	-.883**	.884**	.792**	-.140	.562
.972**	-.615*	.730**	.736**	-.958**	.969**	.888**	-.267	.654*
.818**	-.549	.427	.610*	-.767**	.757**	.578*	-.379	.469
-.390	.756**	-.173	-.654*	.600*	-.434	-.453	-.227	-.707*
-.914**	.793**	-.614*	-.843**	.967**	-.935**	-.881**	.135	-.794**
.232	-.670*	.002	.470	-.329	.326	.491	.388	.626*
-.861**	.814**	-.581*	-.749**	.973**	-.871**	-.915**	-.062	-.792**
.882**	-.520	.571	.672*	-.831**	.848**	.651*	-.457	.466
B. Diastatic activity (Papain), % _L	-.626*	.671*	.771**	-.918**	.978**	.841**	-.302	.589*
-.232	B. Hours to steep to 44%	-.168	-.783**	.722**	-.661*	-.704*	-.269	-.881**
.160	.304	Malting loss, %	.343	-.704*	.702*	.701*	.189	.281
.039	-.595	.402	M. Wort nitrogen, %	-.716**	.819**	.607*	-.012	.802**
.248	.502	-.192	.073	M. Extract, %	-.931**	-.949**	.084	-.728**
.669*	-.228	.178	.447	-.143	M. Diastatic activity, (Lintner)	.879**	-.234	.690*
.070	-.400	.290	-.136	-.874**	.406	M. Proteolytic activity	.026	.728**
-.189	-.593	.542	.311	-.619*	.095	.510	M. Autolytic diastatic activity	*.300
-.162	-.801**	-.282	.639*	-.547	.386	.467	.630*	M. Starch liquefying activity

Partial correlation coefficient, independent of total nitrogen

[†] denotes that the 1% level and * that the 5% level of significance has been attained.

Intravarietal Correlation Coefficients

In Table I the properties examined are listed along the diagonal axis and are prefixed with "B" or "M" to indicate whether the determination was made on barley or malt. The simple intravarietal correlation coefficients are listed in the upper right half of the table. The figure appearing in any particular section represents the simple correlation coefficient for the property listed on the left in the same line as the figure, and the property listed below in the same column as the figure.

The correlation coefficients were calculated from the means, over all varieties, for each station. Since 12 stations were included in the study, there were 12 pairs of values available for the calculation of each coefficient. Accordingly, there are 10 degrees of freedom and the 5% and 1% levels of significance correspond to values of .576 and .708. In the table a single asterisk denotes that the 5% level, and a double asterisk that the 1% level, of significance is attained.

Partial correlation coefficients, independent of total nitrogen, for the various pairs of properties, are listed in the lower left half of Table I. These will be discussed later. However, it may be noted, in passing, that for these correlations nine degrees of freedom are available and the 5% and 1% levels of significance correspond to values of .602 and .735, respectively.

Returning to the simple correlation coefficients it will be found that of the 153 coefficients listed, 72 are significant to the 1% level, 33 to the 5% level, and 48 fail to attain significance. Of the coefficients that fail to attain significance, 17 involve autolytic diastatic activity*, 13 involve insoluble residue of barley, and 8 involve 1000-kernel weight. These three categories account for all but 10 of the coefficients that fail to attain significance.

Examination of the top line of figures in Table I will show that, with the exception of the three properties mentioned above, total barley nitrogen is significantly correlated with all other properties. If the relations are checked for all the nitrogen fractions, it will be observed that all the simple correlation coefficients for these are similar in sign and magnitude to those for total nitrogen. This shows clearly that changes in environment that result in a regular change in total nitrogen also result in a regular change in nitrogen fractions (1, 10, 11). This regularity principle also appears to apply to the majority of the other barley and malt properties (9, 12-15, 19-21).

The numerical values of the coefficients furnish estimates of the degree to which changes in one property are paralleled by changes in the second property. With increasing nitrogen content of the barley all nitrogen fractions of both barley and malt show a highly significant tendency to increase in a regular manner. This also applies to the enzymatic activities and to a lesser extent to malting loss. On the other hand, the amounts of the principal fractions (barley starch, barley extract, and malt extract) including carbohydrates as

* For details of the methods used for measuring various properties, the reader is referred to earlier papers in this series. These are all listed at the end of this paper.

main constituents tend to decrease in a regular manner as nitrogen increases (9). The relations between these fractions and other properties exhibit a marked similarity to the corresponding relations with nitrogen fractions, but the former are invariably opposite in sign to the latter.

Although a change in environment that produces a change in a certain property also tends to produce a corresponding change in most other properties, certain irregularities almost invariably exist. For certain pairs of properties for which the correlation coefficients exceed .95, these irregularities are obviously small and may result mainly from experimental errors. However, for most pairs of properties, and particularly for those yielding correlation coefficients below .70, it is apparent that the associated effects of changing environment on the two properties are relatively loose. In these instances, it is only by making a very broad generalization that one can speak of parallel trends or a regularity principle.

It appears, however, that there are fundamental relations between certain pairs of properties that are so close that both properties must respond in a related manner to any change in environment. Some light on these relations can be obtained by calculating partial correlation coefficients, independent of total nitrogen. In effect, this procedure removes from consideration that part of the change in environment that is reflected by a change in total nitrogen. There remains that part of the environmental change that has no effect on total nitrogen. In other words, it is as though the correlation study were made with data on samples that, though of equal nitrogen content, were actually grown in different places and were thus presumably exposed to environmental conditions that differed in certain respects, however small. It will thus be apparent that, in some ways, the partial correlation coefficients provide a more stringent test of the associated effects of environment on any pair of properties.

A general examination of the partial correlation coefficients listed in Table I will show that 11 attain the 1% level of significance and 15 attain the 5% level. The corresponding figures for the simple correlation coefficients are 72 and 33.

Although space hardly permits a detailed discussion of all the significant partial correlation coefficients, there follow some general comments on the more important of these. As would be expected, close relations exist between the various carbohydrate fractions. Thus environmental conditions that tend to increase starch and barley extract also tend to increase malt extract and to decrease the insoluble barley residue which consists mainly of cellulose and lignin. On the other hand, these same environmental conditions appear to favour a reduction in proteolytic activity and autolytic diastatic activity. No reasonable explanation of these latter associations has been evolved.

Again, as might well be expected, a fairly close relation exists between salt-soluble barley nitrogen and wort nitrogen of barley, and it appears that a fundamental relation exists between these two properties. It seems surprising

TABLE II

SIMPLE INTERVARIETAL CORRELATION COEFFICIENTS AND CORRESPONDING PARTIAL CORRELATION COEFFICIENTS, INDEPENDENT OF SALT-SOLUBLE NITROGEN, FOR BARLEY AND MALT PROPERTIES

Simple correlation coefficient								
B. Total nitrogen, %	.369	.811**	.238	-.552	-.399	.335	-.401	-.241
.562	B. Glutelin nitrogen, %	-.011	-.471	-.461	-.582*	.677*	-.590*	-.387
.829**	.027	B. Hordein nitrogen, %	.027	-.055	-.060	.033	-.115	.077
—	—	—	B. Salt-soluble nitrogen, %	-.348	.018	-.216	.109	.821**
-.515	-.359	-.048	—	B. 1000-kernel weight	.690*	-.613*	.639*	-.300
-.415	-.651*	-.061	—	.743**	B. Starch, %	-.950**	.983**	.312
.407	.668*	.040	—	-.752**	-.969**	B. Insoluble residue, %	-.969**	-.435
-.442	-.614*	-.119	—	.726*	.987**	-.974**	B. Extract, %	.413
.082	-.001	.095	—	-.206	.521	-.462	.570	B. Wort nitrogen, %
.024	.270	-.142	—	-.522	.145	.222	-.122	.074
.634*	-.022	.552	—	.050	-.240	.210	-.348	-.285
-.457	-.504	.128	—	.381	.634*	.256	.624*	.432
-.100	.459	-.305	—	-.420	-.163	.154	-.050	.395
-.563	-.769**	-.238	—	.714*	.948**	-.934**	.887**	.532
-.318	.171	-.500	—	-.362	-.241	.226	-.150	-.008
-.294	-.146	-.252	—	.163	.457	-.336	.508	.602
-.490	-.032	-.560	—	-.018	.323	-.334	.449	.554
-.415	.054	-.587	—	.227	-.301	-.226	.372	.540

Partial correlation coefficient, independent of salt-soluble nitrogen

** denotes that the 1% level and * that the 5% level of significance has been attained.

TABLE II

SIMPLE INTERVARIETAL CORRELATION COEFFICIENTS AND CORRESPONDING PARTIAL CORRELATION COEFFICIENTS, INDEPENDENT OF SALT-SOLUBLE NITROGEN, FOR BARLEY AND MALT PROPERTIES

Simple correlation coefficient								
.199	.238	-.041	.166	-.381	-.039	.070	-.136	-.103
-.188	.336	-.637*	-.231	-.728**	-.239	-.474	-.381	-.310
.076	.505	.092	-.175	-.200	-.324	-.098	-.350	-.383
.739**	-.740**	.840**	.887**	.452	.727**	.874**	.751**	.727**
-.587*	.289	-.099	-.491	.439	-.486	-.230	-.272	-.107
.085	-.174	.359	-.060	.854**	-.153	.238	.227	.194
-.014	.298	-.048	-.122	-.913**	.006	-.348	-.378	-.309
-.001	-.313	.428	.074	.914**	-.024	.340	.377	.333
.635*	-.717**	.856**	.833**	.642*	.594*	.885**	.825**	.809**
B. Diastatic activity (Papain), %	-.706*	.436	.735**	.288	.904**	.634*	.751**	.750**
-.352	B. Hours to steep to 44%	-.706*	-.806**	-.589*	-.814**	-.823**	-.937**	-.954**
-.505	-.231	Malting loss, %	.748**	.688*	.465	.915**	.700*	.697*
.254	-.483	.013	M. Wort nitrogen, %	.431	.829**	.874**	.849**	.801**
-.076	-.424	.637*	.073	M. Extract, %	.325	.644*	.653*	.592*
.800**	-.596	-.391	.579	-.006	M. diastatic activity (Lintner)	.662*	.856**	.816**
-.030	-.536	.689*	.448	.570	.087	M. Proteolytic activity	.807**	.798**
.441	-.859**	.194	.602	.533	.684*	.473	M. Autolytic diastatic activity	.975**
.460	-.922**	.231	.493	.437	.610*	.399	.946**	M. Starch liquefying activity

Partial correlation coefficient, independent of salt-soluble nitrogen

** denotes that the 1% level and * that the 5% level of significance has been attained.

that the partial correlation coefficients for barley diastatic activity as determined in the presence of papain, and malt diastatic activity (.67) are not higher. It will be observed, however, that the corresponding simple correlation is very high (.98). The explanation appears to be that diastatic activity is controlled mainly by those environmental factors that also control total nitrogen content. When these are removed from consideration, by calculating the partial correlation coefficient independent of total nitrogen, the remaining environmental factors have little influence on diastatic activity and hence the possible relation between values for barley and malt is partially obscured by the greater relative importance of random variations contributed by experimental errors.

The property reported in terms of the number of hours required to steep barley to a moisture content of 44%, shows some interesting associations with other properties. Steeping time appears to be increased by those environmental conditions that tend to increase 1000-kernel weight and starch content, and to decrease insoluble barley residue. Steeping time is obviously affected by kernel size but it also appears to be affected by the texture of the kernel.

It is also interesting to note that steeping time is inversely correlated with the starch liquefying activity of the malt, and that the latter is inversely correlated with the starch content of the barley. Whether environmental conditions that tend to increase starch content also tend to change the texture of the kernel, or the nature of the starch grains, so as to slow down the rate of absorption of water and the rate of enzyme attack, or whether these environmental conditions actually decrease the amounts and activity of the starch liquefying enzyme, is not clear. However, some fairly close association appears to exist between these three properties.

In general, the study of the intravarietal relations between various barley and malt properties shows that those environmental factors that affect total nitrogen content also affect most other properties to a greater or lesser extent. Thus when working with samples of one variety, or of a group of closely similar varieties, a determination of nitrogen content provides considerable information on the general malting qualities of the sample. However, if an attempt were made to predict malting quality from nitrogen content alone, considerable variations would be obtained from expected results for certain properties, since these are obviously controlled to a considerable extent by environmental factors that have little effect on total nitrogen content. Among these various properties, there are certain pairs that obviously respond in an associated manner to changes in environment. In some instances this type of association represents an obvious fundamental relation between the properties concerned. In other instances, the nature of the association is by no means clear and the question of whether or not a fundamental relation exists must await the results of further investigation.



Intervarietal Relations

The simple intervarieta! correlation coefficients for the barley and malt properties examined are listed in the upper right half of Table II. These were calculated from the means, over all stations, for each variety. Since 12 varieties were studied, there were available for each correlation 12 pairs of values, corresponding to 10 degrees of freedom and 5% and 1% points of .576 and .708.

Among the 153 simple correlation coefficients listed in Table II, 43 attain the 1% level of significance, 22 attain the 5% level, and 88 are of negligible magnitude in that they fail to attain this latter level of significance. For reasons that will be discussed later, partial correlation coefficients, independent of salt-soluble barley nitrogen, are given in the lower left half of the table.

The intervarieta! correlations between barley and malt properties result from associated effects of genetic or hereditary factors on various pairs of properties. It is not surprising that these associated effects should differ considerably from the effects of environmental factors, and that appreciable differences should thus exist between intra- and intervarieta! correlation coefficients for corresponding pairs of properties. The most striking differences occur in the coefficients for total nitrogen and nitrogen fractions, and in the coefficients for these and the remaining properties. Whereas most of the intravarieta! correlation coefficients are highly significant (Table I), most of the intervarieta! correlation coefficients, given in Table II, fail to attain a significant level. Thus there is no evidence for intervarieta! regularities in composition between nitrogen fractions (1, 10, 11) or between nitrogen fractions and carbohydrates (9, 21).

On the other hand, as would be expected, close intervarieta! associations exist between starch content, insoluble barley residue, barley extract, and malt extract (9, 15, 21). There is also a definite indication that varieties that tend to show high activity with respect to one type of enzyme also tend to show high activities with respect to other enzymes.

While salt-soluble barley nitrogen shows no significant correlation with total nitrogen, the other barley nitrogen fractions, starch, barley extract, or malt extract, it is closely associated with wort nitrogen of both barley and malt and also with all the enzymatic activities, with malting loss, and with steeping time. It appears that barley varieties that are high in salt-soluble nitrogen tend to be high in enzymatic activity, in wort nitrogen, and in malting loss, and that they also tend to absorb water more rapidly in the steep.

The associations among these various properties that are correlated with salt-soluble nitrogen suggest that hereditary factors that express themselves by controlling salt-soluble nitrogen may also play a part in controlling a number of other barley and malt properties, particularly those associated with enzymatic activities. In order to clarify the associations that appear to exist among the various properties that are correlated with salt-soluble nitrogen, and to bring to light other associations that might be masked by

bilateral associations with salt-soluble nitrogen, the partial correlation coefficients, independent of salt-soluble nitrogen, were calculated for each pair of properties. These partial correlation coefficients are listed in the lower left half of Table II. This calculation removes the effects of those genetic factors that express themselves by controlling salt-soluble nitrogen content. If the remaining genetic factors have associated effects on any pair of properties, there is obtained a significant partial correlation coefficient that provides evidence of a relation, between the properties represented, that is independent of bilateral associations between each of the two properties and salt-soluble nitrogen.

Of the 146 partial correlation coefficients, 14 attain the 1% level of significance, 12 attain the 5% level, and the remainder are not significant. The corresponding figures for the simple correlation coefficients are 43 and 22. An examination of the significant partial correlation coefficients shows that of the 14 that attain the 1% level, 12 are of the same order of magnitude as the simple coefficients, and two represent increases from the 5% level; and of the 12 partial correlation coefficients that attain the 5% level, 3 represent decreases from the 1% to the 5% level, and 4 represent rises from below to above the 5% level.

Among the coefficients that remain relatively unchanged by removal of effects associated with salt-soluble nitrogen, the majority involve associations between carbohydrate fractions, total nitrogen, glutelin nitrogen, and hordein nitrogen, on the one hand, and certain other fractions, on the other hand. In general, it appears that genetic factors that control these properties have little influence on salt-soluble nitrogen.

Where there exist appreciable differences between the magnitudes of the simple and partial correlation coefficients, the properties involved are mainly enzymatic activities, wort nitrogen, malting loss, and steeping time. Inter-varietal associations between each of these properties and salt-soluble nitrogen obviously exist, but whereas the simple correlation coefficients suggest that associations exist among all these properties, the partial correlation coefficients show that real associations, independent of common associations, with salt-soluble nitrogen, only exist in fact for certain pairs of these properties. In this category may be classed the following pairs of properties:—diastatic activities of barley and malt; malting loss and proteolytic activity; autolytic diastatic activity and steeping time, and diastatic activity of malt; starch liquefying activity and each of the two last named properties; and starch liquefying activity and autolytic diastatic activity. Fundamental relations appear to exist between these pairs of properties, so that, with respect to each pair, certain genetic factors that control one property also appear to control the other and varieties that are high in one property thus tend to be high (or low if the relation is inverse) in the other property.

For certain other pairs of properties, e.g., proteolytic activity and diastatic activity of barley or malt, the apparent association suggested by a significant simple correlation coefficient, turns out to be a superficial reflection of the

associations that exist between each property and salt-soluble nitrogen. Accordingly, the partial correlation coefficient, independent of salt-soluble nitrogen, fails to attain significance. In these instances it seems safe to assume that certain genetic factors are common to the control of salt-soluble nitrogen and one member of the pair of properties in question; that these factors or others are also common to the control of salt-soluble nitrogen and the second property of the pair; and that there are few or no genetic factors that do not control salt-soluble nitrogen but are common to the control of both properties forming the pair under consideration. Under these conditions, it would appear that between each pair of properties of this type (i.e., giving a significant simple correlation coefficient but failing to give a significant partial correlation coefficient, independent of salt-soluble nitrogen) there exists no fundamental relation, nor any appreciable association other than that which each property has with salt-soluble nitrogen.

Calculation of Additional Correlation Coefficients

The simple correlation coefficients given in Tables I and II can be used to calculate a very large number of other correlation coefficients that may serve to shed additional light on certain of the inter- and intravarietal associations that exist between various barley and malt properties. Some of the more interesting of these associations have been discussed in greater detail in earlier papers in this series, but it seems probable that other investigators may be interested in certain associations that we have not examined. The statistics given in Tables I and II may prove useful for these purposes.

The partial correlation coefficient for any two of the properties listed, independent of any third property, can be calculated from the following formula:—

$$r_{12.3} = \frac{r_{12} - r_{13} \cdot r_{23}}{\sqrt{(1 - r_{13}^2)(1 - r_{23}^2)}}$$

where $r_{12.3}$ is the partial correlation coefficient for Properties 1 and 2, independent of Property 3; and r_{12} is the simple correlation for Properties 1 and 2, etc. Since all the simple correlation coefficients were calculated from 12 pairs of values, there are 9 degrees of freedom for partial correlation coefficients involving 3 variables, and the 5% and 1% points are therefore .602 and .735.

The simple and partial correlation coefficients can also be used to calculate multiple correlation coefficients. For coefficients involving three variables the following equation may be used:—

$$1 - R_{1.23}^2 = (1 - r_{12}^2)(1 - r_{13.2}^2)$$

where $R_{1.23}$ is the multiple correlation coefficient for Property 1 with Properties 2 and 3, and r_{12} and $r_{13.2}$ are the appropriate simple and partial correlation coefficients. There are nine degrees of freedom for the multiple correlation coefficient involving three variables and the 5% and 1% points are .697 and .800. It should be noted that with the data presented in this paper, it is

not possible to determine whether the multiple correlation coefficient is significantly greater than the corresponding simple correlation coefficient. Experience must serve as a guide in assessing the usefulness of adding the second independent variable. Multiple correlation coefficients involving more than three variables can also be calculated but the calculations become tedious and will hardly be worth undertaking in view of the limited number of degrees of freedom.

Generally speaking, the intravarietal correlation coefficients, given in Table I or calculated from these statistics, will be of most interest to the practical maltster. In almost all countries single varieties or mixtures of closely related varieties are used for malting purposes. Thus the practical maltster is interested primarily in the effects of environment on malting quality and in the relations between various properties that exist within one variety or closely related group of varieties.

The intervarietal correlation coefficients, given in Table II or calculated from these statistics, will be of more interest to the plant breeder and to the cereal chemist who is assisting in the development of new malting barleys. With a thorough knowledge of the intervarietal relations and associations that exist between barley and malt properties, it is possible to form a fairly reliable judgment of the comparative malting qualities of new varieties from the results of analysis of the barley. In the early stages of a breeding program it should be possible to do without the malting test, which requires so much time and special equipment, without danger of discarding new lines with superior malting qualities. Experience has already shown that by a judicious use of barley analysis and malting tests, fortified by a knowledge of the intervarietal relations between barley and malt properties, the malting chemist can assist the plant breeder materially in his task of developing superior varieties of malting barley.

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THE PHYTOPLANKTON OF SOUTHERN AND CENTRAL SASKATCHEWAN

PART I¹

BY PAUL E. KUEHNE²

Abstract

Plankton collections from 58 lakes of the Prince Albert Park and the southern half of Saskatchewan yielded 292 species and varieties of algae, among them *Chaetoceros Elmorei*, *Nodularia spumigena*, and a new variety of *Lyngbya Birgei*. The lakes, chemically alkaline, are classified as freshwater and saline, and the various classes of algae are briefly discussed with reference to these two types of lakes. A list of the species collected is given, showing localities and relative abundance of each type.

Phycological literature offers very little on the algal flora of Saskatchewan: a paper on the diatoms of the Quill lakes (1), the names of several other algae from the same lakes (14), and some occasional references to those of Carlyle Lake (17). Yet, the algae, and especially the plankton algae, play a vital role in aquatic ecology. They are, to paraphrase H. G. Wells, "the base of the lake's vital pyramid, on which are supported almost all our food fishes". To contribute to a knowledge of the algal flora is the purpose of this paper.

The material was collected by Dr. D. S. Rawson in nine lakes and two creeks of the Prince Albert Park in the years 1928-31, and by J. E. Moore, in the years 1938 and 1939, in 47 lakes (Fig. 1) scattered throughout the southern half of the province. Detailed chemical and physical studies of these lakes have been made by the collectors (21, 22, 28). All lakes have a pH value above 7 and are, therefore, alkaline in the strictly chemical sense. But for limnological reasons, they are divided into two groups, saline (popularly called "alkaline") and freshwater. This classification is based upon the total solid content of the waters. Lakes with a concentration of more than 300 mg. of solids per litre of water (or, as usually stated, of more than 300 p.p.m.) are considered saline, and those with less than 300 p.p.m. as freshwater. On this basis, the lakes of the Prince Albert Park are freshwater, whereas all but five of those studied by Mr. Moore belong to the saline group (Table I). Dr. Rawson and Mr. Moore generously permitted the study of their collections and were ever ready to supply requested information. The author takes this opportunity of expressing to them his sincere thanks.

No new species are described in this paper, but a new variety of *Lyngbya Birgei* has been established. Many of the forms listed have previously been reported from adjacent regions, and almost all of them occur in North America. Some are included as doubtful because of insufficient or poorly preserved material. With few exceptions, motile forms have been excluded. Several collections, for instance, contained *Chlamydomonas*, *Euglena*, and other forms

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FIG. 1 Sketch map showing distribution of lakes in southern Saskatchewan. By J. E. Moore.

apparently motile when alive, but their state of preservation did not permit specific identification. Filaments, representing several species of *Spirogyra*, were found in many collections, but all in the sterile condition.

The collections from Little Manitou Lake contained much material of a minute, branching alga (Figs. 2 and 3). The general vegetative characteristics, such as mode of branching, diameter of the cell, etc., pointed to that strange Phaeophycean, *Pleurocladia lacustris*, which has been reported only from lakes in northern Germany (24). Some specimens, however, showed akinete-like structures, unknown in that brown alga. Dr. W. R. Taylor*, to whom the material was submitted, at first shared this opinion. In a later communication he suggested that the plant might be *Ctenocladus*, a brackish Chlorophycean, found in some pools near Mariana, California, in 1929 (32). This alga has cell series rather like the "akinetes" of the Saskatchewan specimens, but is lacking in other characters. Dr. Taylor rightly added, "So much of the point of view depends on the color!" Only fresh specimens, promptly

* Personal communication.

TABLE I

TOTAL NUMBER OF SPECIES AND VARIETIES AND OTHER DATA FOR EACH LAKE

Lakes	Max. depth, ft.	Total solids, p.p.m. ¹	Number of species					Dates	Collecting methods ²		
			Cyanophyceae	Chlorophyceae	Bacillariaceae	Miscellaneous ³	Total		ST	TV	STL
A. Saline											
Antelope	28	13,640	8	6	4	0	18	7/30/38 8/12/39	*	*	*
Atten	27	1394	3	2	19	3	27	5/31/39	*	*	*
Basin	35	11,805	7	7	4	2	20	6/30/39	*	*	*
Big Quill	20	30,022	11	5	8	3	27	9/ 2/38 7/ 3/39	*	*	*
Birch	13	636	20	12	14	4	60	6/15/39 8/ 1/39	*	*	*
Bitter	55	13,981	7	0	19	2	28	5/26/39	*	*	*
Brightsand	22	452	2	7	43	3	55	6/ 9/39 7/30/39	*	*	*
Carlyle	50	1341	2	1	4	1	8	7/ 1/38	*	*	*
Christopher	33	542	10	8	20	5	43	6/27/39	*	*	*
Clearwater	32	781	6	9	4	2	21	7/27/38	*	*	*
Echo	57	1231	16	17	39	1	73	6/23/38 5/20/39 7/ 7/39	*	*	*
Edwards	13	894	4	1	1	3	9	8/30/39 7/15/38	*	*	*
Emma	30	528	12	5	24	4	45	6/27/39	*	*	*
Fishing	35	3183	21	10	25	4	60	7/10/38 7/ 1/39	*	*	*
Good Spirit	13	868	11	4	6	1	22	7/ 5/38	*	*	*
Greenwater	25	357	17	9	16	3	45	7/13/38 7/ 1/39	*	*	*
Jackfish	15	1172	17	20	34	2	73	8/11/38 5/29/39 7/19/39	*	*	*
Kenderdine	38	1013	22	27	25	5	79	8/20/38 6/ 2/39 7/22/39	*	*	*
Kenossee	25	1186	3	1	6	1	11	6/29/38	*	*	*
Last Mountain	80	2416	24	21	32	4	81	5/14/38 5/18/39 7/ 5/39	*	*	*
Lenore	23	5486	11	12	6	1	30	8/29/39 7/17/38	*	*	*
Little Loon	33	352	8	3	18	2	31	6/14/39	*	*	*
Little Manitou (north of Watrous)	12	115,500	2	5	5	1	13	5/16/39 7/ 4/39	*	*	*
Little Quill	5	21,387	10	6	15	3	34	8/28/39 9/ 2/38 7/ 2/39	*	*	*
Madge	43	412	11	5	7	1	24	7/ 8/38	*	*	*
Manito	75	19,233	3	5	7	3	18	8/17/38 5/10/39 5/31/39 6/ 1/39 7/24/39	*	*	*
Margo	11	1497	15	13	18	1	47	7/10/38 7/ 2/39	*	*	*

TABLE I—Continued

TOTAL NUMBER OF SPECIES AND VARIETIES AND OTHER DATA FOR EACH LAKE—Continued

Lakes	Max. depth, ft.	Total solids, p.p.m. ¹	Number of species					Dates	Collecting methods ²		
			Cyanophyceae	Chlorophyceae	Bacillariaceae	Miscellaneous ³	Total		ST	TV	STL
A. Saline—concluded											
Midnight	10 ²	448	11	9	10	2	32	7/31/39			*
Murray	19	751	22	13	30	5	70	8/12/38	*	*	
								5/29/39	*	*	
								6/ 7/39			*
								8/17/39	*	*	*
								8/18/39	*		
Pasqua	60 ³	995	11	9	16	0	36	7/ 8/39			*
Pelletier	27	461	9	7	6	3	25	7/29/38	*	*	
Redberry	80	13,666	10	12	12	2	36	8/25/38	*	*	
								6/17/39	*	*	*
								8/ 7/39	*	*	
Round I (Qu'Appelle Valley)	38	887	10	3	4	3	20	6/28/38	*	*	
Round II (northwest of Prince Albert)	20	1307	7	5	11	2	25	6/23/39	*	*	*
Sandy Beach	40 ⁴	1547	2	2	7	1	12	7/23/39			*
Soda	7	9318	0	5	9	3	17	6/ 2/39			*
								7/21/39	*	*	*
Stoney (south of Humboldt)	17	6787	6	2	3	1	12	7/18/38	*	*	
Stony (northwest of Glaslyn)	10	412	19	13	15	4	51	8/ 3/39	*	*	*
Sturgeon	27	486	14	3	15	4	36	6/24/39	*	*	*
Wakaw	30	2915	11	2	19	0	32	5/23/39	*	*	*
Wilson	22	907	12	6	1	0	19	8/ 8/38	*	*	
Witcheakan	10 ³	838	10	13	9	1	33	8/ 4/39			*
Total—Saline lakes			51	81	103	11	246				
B. Freshwater											
Bagwa (P. A. Park)	?	?	11	0	2	0	13	9/ 3/28	*		
Beastrap Creek (P. A. Park)	?	?	0	5	6	1	12	8/13/28	*		
Broughton	17	171	15	8	11	2	36	8/ 2/39	*	*	*
Crean (P. A. Park)	89	?	10	8	21	4	43	6/15/31		*	
								7/23/31		*	
								8/24/31		*	
								9/17/31		*	
Deep	78	295	1	3	25	2	31	5/25/39	*	*	*
Halkett (P.A. Park)	173	283	17	18	18	4	57	7/28/30	*	*	
								9/ 3/30	*	*	
								8/11/31		*	
Helene	18 ³	217	10	14	34	2	60	6/15/39	*	*	*
Kingsmere (P. A. Park)	154	?	14	12	14	5	45	6/28/28	*	*	
								8/28/28	*	*	
								9/ 3/28	*		
								6/ 8/31		*	
								7/17/31		*	
								8/18/31		*	
								9/14/31		*	

TABLE I—*Concluded*TOTAL NUMBER OF SPECIES AND VARIETIES AND OTHER DATA FOR EACH LAKE—*Concluded*

Lakes	Max. depth, ft.	Total solids, p.p.m. ¹	Number of species					Dates	Collecting methods ²		
			Cyanophyceae	Chlorophyceae	Bacillariaceae	Miscellaneous ³	Total		ST	TV	STL
B. Freshwater											
— <i>concluded</i>											
Lavallee											
(P. A. Park)	46	?	9	6	7	2	24	7/29/29	*		
Meeting	43	231	18	16	16	2	52	7/ 5/39	*	*	*
Moose Creek											
(P. A. Park)	?	?	11	7	7	2	27	7/27/28	*		
Shady (P. A. Park)	?	?	13	6	3	1	23	8/21/28	*		
Tibiska (P.A. Park)	44	?	9	7	6	3	25	8/ 2/29	*		
Turtle	45	261	18	19	37	2	76	6/12/39	*	*	*
								7/27/39	*	*	*
Wabeno											
(P. A. Park)	?	?	4	7	10	4	25	9/ 7/29	*		
Waskesiu											
(P. A. Park)	80	178	34	52	25	5	116	7/ 3/28		*	
								7/27/28	*	*	
								8/ 3/28	*		
								8/13/28	*	*	
								8/17/28	*	*	
								9/ 7/28	*	*	
								9/15/28	*		
								7/ 2/29		*	
								7/ 8/29	*		
								8/30/29	*		
								6/29/31		*	
								7/20/31	*	*	
								8/ 2/31		*	
								9/ 1/31		*	
Waskesiu											
(Narrows)			8	0	1	0	9	9/10/31	*		
Total—Freshwater lakes			43	75	71	8	197				
Total—All lakes			57	112	111	12	292				

¹ Total solid content of water in parts per million (cf. text).² ST = surface tow; TV = total vertical haul; STL = surface tow in littoral region.³ Estimated value; not measured at time of collecting.⁴ Reported value; not measured at time of collecting.⁵ *Heterokontae*, *Chrysophyceae*, *Dinophyceae*.

examined, can furnish that information. Furthermore, a knowledge of the various reproductive structures and of the life history are very desirable. Such investigations are planned.

In Table I are given the number of species and varieties collected in each lake and the total for all the lakes. Even with the inclusion of all forms omitted from this discussion, the plankton flora of the Saskatchewan lakes can scarcely be regarded as rich. The following considerations may account for this apparent poverty.

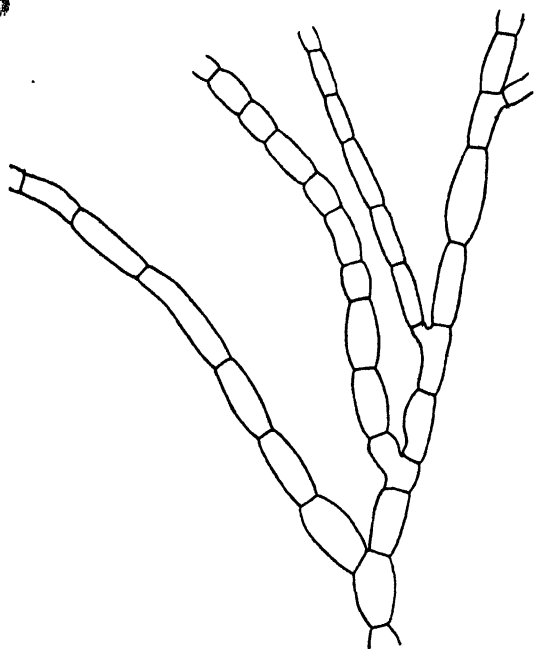


FIG. 2. Vegetative cells of plant found in Little Manitou Lake. 247X.

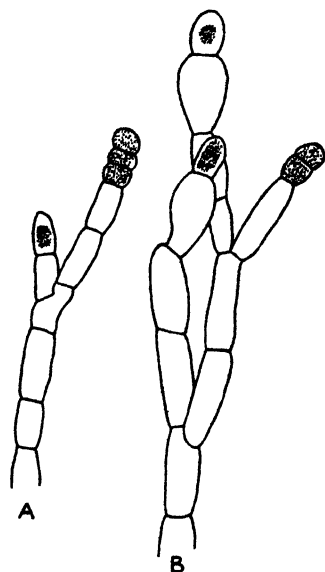


FIG. 3. Akinete-like terminal cells of plant found in Little Manitou Lake. Type A more frequent than Type B. 247X.

All collections were made with plankton nets of No. 20 silk bolting cloth. This mesh allows most of the minute organisms, such as *Ankistrodesmus*, *Oocystis*, *Crucigenia*, *Cocconeis*, and *Cyclotella* spp., to pass through the net. To obtain them in appreciable quantities, if at all, the lake water would have to be centrifuged, though a cotton disk serves as a fairly efficient substitute (3). No samples were taken by these methods, because the original investigations were primarily ecological, not systematic.

For most of the saline lakes and for some of the freshwater type, two methods of collecting were employed, the surface tow and the total vertical haul. "In the first case the net was towed behind the boat, which was rowed slowly, for a period of three minutes. In making a vertical haul the net was pulled up from the bottom of the lake at the rate of approximately one-half metre per second" (21). Blue-green and green algae were usually more numerous in the surface tow, and the diatoms in the vertical haul; in most cases, however, the difference was not very striking. This, no doubt, is due to the shallowness of most lakes and, in the deeper lakes, perhaps, to a narrow epilimnion at the time of collecting.

Most lakes are represented by collections made in a single day. Yet, as is well known, owing to such variable factors as temperature, intensity of light, supplies of nitrates, phosphates, and other dissolved nutrient materials, presence of predators, etc., the plankton constitutes an ever changing popu-

TABLE II

OCCURRENCE OF THE MORE COMMON ALGAE OF WASKESIU LAKE

Species	1928										1929			1931				
	July			Aug.						Sept.		July		Aug.	June	July	Aug.	Sept.
	3	27	27	3	13	13	17	17	7	15	2	8	30	29	20	20	8	1
	TV	ST	TV	ST	ST	TV	ST	TV	TV	ST	TV	ST	ST	TV	ST	TV	TV	TV
Cyanophyceae:																		
<i>Anabaena</i>																		
<i>circinalis</i>	1*	1	-	-	1	-	2	-	1	1	-	-	3	2	-	1	-	2
<i>Lemmermanii</i>	1	3	2	4	1	-	3	2	2	2	1	1	3	2	2	1	-	2
<i>spiroides</i>	-	-	-	-	-	-	-	1	2	-	-	1	3	-	-	1	-	-
<i>Lyngbya</i>																		
<i>limnetica</i>	1	1	1	-	-	2	1	-	-	-	2	1	1	4	-	5	4	2
<i>Coelosphaerium</i>																		
<i>Nacelianum</i>	1	2	1	3	3	1	3	1	2	3	-	2	3	1	3	2	-	1
<i>Aphanocapsa</i>																		
<i>pulchra</i>	1	1	1	1	2	-	2	-	2	2	1	2	2	-	-	-	2	2
<i>elachista</i> var. <i>conferta</i>	2	1	-	-	1	-	3	2	2	3	-	1	2	-	1	2	1	-
<i>Microcystis</i>																		
<i>aeruginosa</i>	-	1	-	1	1	1	2	-	-	3	1	2	3	1	-	2	-	2
Chlorophyceae:																		
<i>Pediastrum</i>																		
<i>Boryanum</i>	2	2	2	2	2	1	1	1	2	1	2	3	2	2	2	2	2	2
<i>duplex</i>	-	2	-	2	1	-	-	-	-	-	1	2	1	2	2	1	-	-
<i>duplex</i> var. <i>clathratum</i>	-	1	-	2	2	1	1	-	2	-	2	2	2	1	1	1	-	1
<i>Staurastrum</i>																		
<i>paradoxum</i>	2	-	2	-	-	-	-	-	1	1	1	1	-	-	2	-	1	-
<i>longiradiatum</i>	-	2	-	1	2	1	1	2	1	1	1	1	-	-	-	1	2	-
<i>Sphaerocystis</i>																		
<i>Schroeteri</i>	-	-	-	-	3	2	3	1	1	2	1	1	2	-	1	1	-	1
<i>Dictyosphaerium</i>																		
<i>pulchellum</i>	-	-	-	-	-	-	-	-	1	-	3	2	1	-	-	2	1	1
Bacillariaceae:																		
<i>Melosira</i>																		
<i>granulata</i>	4	3	2	2	1	2	2	2	3	4	5	4	1	4	2	5	3	4
<i>crenulata</i>	3	3	-	-	2	-	-	2	2	2	2	-	-	1	1	1	2	-
<i>Stephanodiscus</i>																		
<i>niagarae</i>	1	2	-	3	2	2	3	2	3	4	3	3	2	4	3	3	2	3
<i>Fragilaria</i>																		
<i>crotonensis</i>	4	3	3	-	-	2	3	1	3	3	3	4	1	3	3	2	2	2
<i>capucina</i>	2	-	2	-	-	-	-	1	1	-	3	2	1	2	1	1	-	-
<i>Tabellaria</i>																		
<i>fenestrata</i>	-	1	1	-	-	1	1	1	1	-	2	2	-	1	-	1	2	2
<i>Asterionella</i>																		
<i>formosa</i>	-	1	1	-	-	1	1	-	2	1	2	-	2	-	-	-	-	2
Miscellaneous:																		
<i>Dinobyron</i>																		
<i>divergens</i>	3	2	2	-	-	1	-	2	-	2	2	-	-	2	2	-	3	-
<i>Ceratium</i>																		
<i>hirundinella</i>	3	4	3	4	4	3	4	2	3	2	1	2	1	-	3	1	2	3

NOTE: "ST" and "TV" after the dates denote surface tow and total vertical haul, respectively (cf. text).

* These numbers indicate the relative abundance in the sample: 1 = rare, 5 = very abundant.

tion. Some forms are present during one month, scarce during the next, and back again during the third when environmental conditions have again become favourable (Table II). To obtain a fairly representative picture of the plankton flora of a given lake, collections should be made on several days, distributed throughout the year. The results from the lakes in which more than one collection was made may be offered in confirmation of this; in Echo Lake 73 species were collected in four days; in Kenderdine 79 species in three days; in Last Mountain 81 species in four days; and in Waskesiu 116 species in 14 days.

Owing to the short summer in the Saskatchewan region, most of the algae have only a single maximum. Ricker (29) and others, however, found that many diatoms have two; but, since they come early and late in the year, the Saskatchewan collections did not show this. Oltmanns (23) states that in northern regions *Ceratium* is likewise bimodal. This was found to be true only in Kingsmere Lake, though the collections from Waskesiu for 1931 also demonstrated this to some degree.

In one year only (1939) samples were taken also from the littoral regions. Plankton catches are usually restricted to the open water, because many of the littoral forms are not true *plankton* organisms; that is, organisms that "remain suspended in the water during their entire existence". Certain algae, however, such as *Oedogonium*, *Spirogyra*, *Ulothrix*, and *Cladophora*, are epiphytic in their early stages, and only become free floating tangles later in the season, bearing epiphytes and sheltering unicellular and colonial organisms. They may then drift into the open water and thus form part of the plankton. Economically they are, of course, important because, "the young fish of very many species live in this habitat and are almost wholly dependent upon the small organisms as a food supply following the absorption of the yolk sac" (2). No such floating tangles were encountered, but the littoral collections yielded a large number of species, especially diatoms, that otherwise would have been left unrecorded.

A List of the Plankton Algae Collected in Southern and Central Saskatchewan

The scheme of classification in the following list is that used in Smith's *Fresh-water Algae of the United States* (32), the only book that describes all the genera known to occur in the United States and probably also in the southern parts of Canada. For each species, the numbers 1 to 5 after the name of each lake indicate the relative abundance of that particular form in the collections: 1, rare; 2, occasional; 3, common; 4, abundant; 5, very abundant. The lakes that are represented by more than one collecting day have two numbers, to show the range. For these, the figure "0" implies that on some days no specimens were found.

After each specific name are listed one or more authors who give a complete and authoritative description of that form and, in most instances, satisfactory illustrations. Boyer (5) shows no illustrations but refers to numerous sources

of information. For the desmids two references are usually cited, (34) and (31), though in many instances the dimensions of the Saskatchewan specimens come only within the limits given in the first. This is especially true of the various *Closterium* species.

The content of the list is summarized as follows:

Classes	Families	Genera	Species and varieties
Cyanophyceae	5	19	57
Heterokontae	3	3	3
Chrysophyceae	1	1	4
Bacillarieae	12	34	111
Chlorophyceae	16	40	112
Dinophyceae	1	3	5
Total	38	100	292

The Cyanophyceae and Chlorophyceae are discussed in Part I of this paper.

CYANOPHYCEAE

The blue-green algae are well represented in both types of lakes and form the dominant organisms during the warmest months of the year. *Anabaena*, *Aphanizomenon*, *Gloeotrichia*, and *Microcystis* are absent in the highly saline lakes. In some of the less saline lakes, however, they may become so abundant as to form a "water bloom". Such blooms usually consist of several genera with one or two species dominating. The following lakes showed a more or less heavy bloom of certain algae.

Bagwa: *Anabaena*, *Aphanizomenon*, *Lyngbya*.

Greenwater: *Anabaena*, *Aphanizomenon*, *Gloeotrichia*.

Margo: *Gloeotrichia*, *Microcystis*.

Murray: *Anabaena*, *Microcystis*.

Shady: *Aphanizomenon*, *Anabaena*, *Volvox*.

Waskesiu Narrows: *Anabaena*, *Aphanizomenon*, *Microcystis*.

Moose Creek, near its entrance into Waskesiu, also had a heavy bloom of *Anabaena* spp. with some *Microcystis* and *Aphanizomenon*. In Antelope Lake, *Chroococcus minutus* was so abundant in 1938 as to almost constitute a bloom.

CHROOCOCCACEAE

Chroococcus dispersus (v. Keiss.) Lemm. (30, p. 28, Pl. 1, Fig. 2; 11, p. 84, Fig. 84).

Big Quill (0-3), Bitter (1), Broughton (2), Fishing (0-1), Halkett (0-1), Helene (1), Jackfish (0-1), Kenderdine (0-1), Kingsmere (0-1), Last Mountain (0-2), Lenore (1), Pelletier (2), Waskesiu (0-2).

Chroococcus dispersus* var. *minor G. M. Smith (30, p. 28, Pl. 1, Fig. 3; 11, p. 84).

Big Quill (0-2), Last Mountain (0-2), Little Quill (0-1), Manito (0-1), Meeting (1), Stony (2), Turtle (0-1), Waskesiu (0-1).

Chroococcus limneticus Lemm. (30, p. 29, Pl. 1, Fig. 4; 11, p. 82, Fig. 82; 10, p. 40, Fig. 44).

Antelope (0-1), Big Quill (0-1), Birch (3-4), Bitter (1), Brightsand (1-2), Broughton (2), Christopher (2), Echo (0-3), Emma (3), Fishing (0-2), Good Spirit (1), Greenwater (1-2), Halkett (0-1), Helene (2), Jackfish (0-3), Kenderdine (0-1), Last Mountain (0-2), Little Loon (3), Meeting (2), Midnight (3), Moose Creek (1), Murray (2-3), Pelletier (2), Redberry (0-1), Sandy Beach (1), Stoney (2), Stony (3), Sturgeon (2), Tibiska (2), Turtle (1-3), Wakaw (1), Waskesiu (0-2), Witchehan (1).

Chroococcus limneticus var. **carneus** (Chod.) Lemm. (30, p. 30, Pl. 1, Fig. 6; 11, p. 84).
Last Mountain (0-1).

Chroococcus limneticus var. **distans** G. M. Smith (30, p. 30, Pl. 1, Fig. 7; 11, p. 84).
Fishing (2), Last Mountain (0-1), Meeting (1), Waskesiu (0-2).

Chroococcus limneticus var. **subsalsus** Lemm. (30, p. 29, Pl. 1, Fig. 5; 11, p. 84).

Big Quill (2), Birch (3), Christopher (1), Clearwater (1), Emma (2), Fishing (1), Good Spirit (2), Greenwater (0-1), Jackfish (0-1), Kenderdine (0-1), Lavallee (2), Lenore (1), Little Loon (1), Meeting (2), Midnight (2), Stony (3), Sturgeon (3), Turtle (0-2), Waskesiu (0-2), Wilson (2), Witchehan (3).

Chroococcus minutus (Kütz.) Näg. (30, p. 28, Pl. 1, Fig. 1; 11, p. 79, Fig. 74).

Antelope (3-4), Broughton (1), Crean (0-1), Echo (0-4), Halkett (0-2), Jackfish (0-1), Kenderdine (0-2), Kingsmere (0-1), Last Mountain (0-2), Madge (2), Margo (0-2), Sandy Beach (2), Stony (3), Wabeno (2), Waskesiu (0-2).

Chroococcus turgidus (Kütz.) Näg. (30, p. 31, Pl. 1, Fig. 9; 11, p. 77, Fig. 71).

Birch (3-1), Fishing (0-1), Last Mountain (0-1), Pelletier (2), Round II (1), Stony (2), Waskesiu (0-2).

Aphanocapsa delicatissima W. and G. S. West (30, p. 41, Pl. 2, Fig. 7; 11, p. 65, Fig. 54).

Big Quill (0-2), Bitter (2), Good Spirit (1), Jackfish (0-1), Kenderdine (0-2), Last Mountain (0-3), Little Quill (2-3), Murray (0-1), Waskesiu (0-3), Wilson (2).

Aphanocapsa elachista var. **conferta** W. and G. S. West (30, p. 42, Pl. 2, Fig. 3; 11, p. 65, Fig. 56; 10, p. 21, Fig. 21).

Birch (0-1), Christopher (1), Clearwater (3), Crean (0-1), Echo (0-2), Good Spirit (2), Greenwater (0-2), Halkett (0-2), Helene (1), Jackfish (1-2), Kenderdine (1-4), Lenore (2), Madge (2), Midnight (2), Moose Creek (1), Murray (0-1), Pelletier (1), Redberry (0-2), Round I (2), Stoney (1), Stony (3), Tibiska (2), Turtle (1-2), Wabeno (2), Waskesiu (0-3).

Aphanocapsa Grevillei (Hass.) Rab. (30, p. 43, Pl. 3, Fig. 1; 11, p. 65, Fig. 57).

Antelope (0-2), Bagwa (2), Birch (2-3), Halkett (0-2), Madge (2), Stony (2), Tibiska (2), Waskesiu (0-3).

Aphanocapsa pulchra (Kütz.) Rab. (30, p. 42, Pl. 2, Fig. 9; 11, p. 65, Fig. 53).

Birch (2), Broughton (2), Christopher (2), Crean (0-2), Echo (0-1), Edwards (1), Emma (2), Fishing (1), Greenwater (2-3), Jackfish (0-1), Kenderdine (1-2), Kingsmere (0-1), Last Mountain (0-3), Lavallee (2), Little Loon (2), Little Quill (0-1), Madge (2), Margo (2), Meeting (1), Midnight (2), Moose Creek (2), Murray (0-4), Pasqua (2), Pelletier (1), Round I (2), Round II (3), Stony (3), Sturgeon (2), Tibiska (1), Turtle (0-2), Wakaw (2), Waskesiu (0-2), Wilson (1).

Aphanocapsa rivularis (Carm.) Rab. (30, p. 43, Pl. 3, Fig. 2; 11, p. 66, Fig. 58).

Echo (0-3), Greenwater (0-1), Meeting (1), Murray (0-1), Waskesiu (0-1).

Microcystis aeruginosa Kütz. (30, p. 39, Pl. 5, Figs. 2, 3; 11, p. 58, Figs. 37, 40).

Bagwa (1), Birch (2-3), Broughton (3), Christopher (2), Echo (1-3), Edwards (1), Emma (3), Fishing (2), Good Spirit (2), Greenwater (1-3), Helene (1), Jackfish (0-2), Kenderdine (2-3), Last Mountain (2-3), Lenore (1), Little Loon (3), Madge (2), Margo (3), Meeting (2), Midnight (2), Moose Creek (2), Murray (2-4), Pasqua (2), Pelletier (2), Round I (1), Round II (2), Shady (3), Stoney (2), Stony (3), Sturgeon (3), Turtle (2), Wakaw (2), Waskesiu (0-3), Waskesiu Narrows (3), Witchehan (1).

Microcystis aeruginosa var. **major** (Wittr.) G. M. Smith (30, p. 40, Pl. 4, Fig. 6; 11, p. 60).

Birch (2-3), Broughton (1), Echo (1-4), Fishing (1), Jackfish (0-3), Kenderdine (2), Moose Creek (2), Murray (0-2), Round II (2), Sturgeon (2), Wakaw (2), Waskesiu (0-2), Wilson (1).

- Microcystis flos-aquae** (Wittr.) Kirchner (30, p. 39, Pl. 5, Fig. 1; 11, p. 60, Figs. 41, 42).
 Birch (1-2), Broughton (2), Echo (0-2), Emma (2), Fishing (1-2), Greenwater (0-3), Halkett (0-1), Helene (1), Jackfish (0-1), Kenderdine (0-2), Last Mountain (0-1), Lavallee (1), Lenore (2), Madge (1), Margo (1), Meeting (2), Murray (0-3), Pasqua (2), Redberry (0-1), Shady (1), Stony (3), Sturgeon (1), Turtle (2), Waskesiu (0-2), Waskesiu Narrows (2).
- Microcystis pulvera** (Wood) Migula (30, p. 40; 11, p. 62).
 Birch (1-2), Emma (1), Fishing (0-1), Kenderdine (0-1), Midnight (1), Murray (0-1), Pasqua (1), Stony (2), Turtle (0-2), Waskesiu (0-1).
- Microcystis pulvera** var. *incerta* (Lemm.) Crow (= *M. incerta* Lemm.) (30, p. 40, Pl. 5, Fig. 4; 11, p. 65, Fig. 47; 10, p. 20).
 Birch (0-2), Echo (0-1).
- Mycrocystis viridis** (A. Br.) Lemm. (11, p. 58; 10, p. 18, Fig. 13).
 Bagwa (1), Shady (1), Wilson (1).
- Merismopedia elegans** A. Br. (30, p. 32, Pl. 2, Fig. 5; 11, p. 107, Fig. 126; 10, p. 13, Fig. 11).
 Atten (1), Bitter (1), Broughton (1), Halkett (0-1), Margo (0-1), Murray (0-1), Round II (1), Wakaw (1).
- Merismopedia glauca** (Ehr.) Näg. (30, p. 32, Pl. 2, Fig. 4; 11, p. 106, Fig. 125).
 Basin (1), Bitter (1), Broughton (1), Echo (0-1), Kenderdine (0-2), Kingsmere (0-1), Last Mountain (0-3), Margo (0-1), Pasqua (1), Shady (1), Turtle (0-1), Wakaw (1), Witchekan (1).
- Merismopedia punctata** Meyen (30, p. 33, Pl. 2, Fig. 3; 11, p. 106, Fig. 124).
 Atten (1), Basin (1), Big Quill (0-2), Clearwater (1), Fishing (0-1), Greenwater (0-2), Halkett (0-1), Last Mountain (0-1), Lenore (1), Meeting (1), Turtle (0-1), Wakaw (1), Waskesiu (0-1), Wilson (1).
- Merismopedia tenuissima** Lemm. (30, p. 33, Pl. 2, Fig. 2; 11, p. 106, Fig. 123).
 Big Quill (0-1), Birch (0-1), Broughton (2), Christopher (2), Emma (1), Helene (1), Kenderdine (0-2), Midnight (1), Murray (0-1), Redberry (0-1), Shady (1), Stony (1), Stony (2), Sturgeon (2), Turtle (1-2), Waskesiu (0-1), Witchekan (2).
- Holopedia irregularis** Lagerh. (11, p. 109; 10, p. 16; 13, p. 162, Fig. 59A).
 Lenore (1), Murray (0-1), Pasqua (1), Witchekan (1).
- Aphanothece stagnina** (Spreng.) A. Br. (30, p. 45, Pl. 6, Fig. 2; 11, p. 70, Fig. 65).
 Bagwa (1), Waskesiu (0-2).
- Coelosphaerium dubium** Grunow (30, p. 35, Pl. 3, Fig. 7; 11, p. 102, Fig. 118).
 Antelope (0-1), Birch (0-2), Christopher (1), Murray (0-1).
- Coelosphaerium Kuetsingianum** Näg. (30, p. 34, Pl. 3, Figs. 4, 5; 11, p. 102, Figs. 116, 117).
 Fishing (0-1), Last Mountain (0-1), Meeting (1), Stoney (1), Stony (1), Turtle (0-1), Waskesiu (0-2), Waskesiu Narrows (1), Wilson (1).
- Coelosphaerium Naegelianum** Unger (30, p. 35, Pl. 3, Fig. 6, Pl. 4, Fig. 1; 11, p. 101, Fig. 115).
 Antelope (4), Basin (1), Birch (3-4), Carlyle (1), Clearwater (1), Echo (0-3), Emma (2), Fishing (0-2), Good Spirit (2), Greenwater (0-1), Helene (2), Jackfish (0-3), Kenderdine (2), Kingsmere (0-1), Last Mountain (2-3), Lavallee (2), Lenore (1), Little Loon (3), Little Quill (0-1), Margo (0-3), Meeting (2), Moose Creek (2), Murray (0-2), Pelletier (1), Redberry (0-1), Round I (3), Shady (2), Stoney (1), Stony (3), Sturgeon (3), Tibiska (2), Turtle (2), Wakaw (1), Waskesiu (0-3), Waskesiu Narrows (3), Wilson (1).
- Gomphosphaeria aponina** Kütz. (30, p. 37, Pl. 4, Figs. 2, 3; 11, p. 98, Figs. 108, 109, 112, 113).
 Antelope (2), Big Quill (0-1), Birch (3-4), Bitter (1), Broughton (3), Christopher (1), Clearwater (1), Echo (0-1), Emma (2), Fishing (0-2), Halkett (0-2), Helene (1), Jackfish (2-3), Kenderdine (0-3), Last Mountain (0-3), Margo (0-3), Meeting (1), Midnight (2), Murray (0-2), Redberry (0-3), Stony (3), Sturgeon (1), Turtle (0-1), Waskesiu (0-1).
- Gomphosphaeria aponina** var. *cordiformis* Wolle (30, p. 37, Pl. 4, Fig. 4; 11, p. 98, Fig. 111).
 Birch (1-2).

Amphosphaeria lacustris Chod. (30, p. 36, Pl. 4, Fig. 5; 11, p. 98, Fig. 110, 114).

Birch (1), Good Spirit (1), Greenwater (0-1), Kenderdine (1-2), Lenore (3), Little Quill (1-2), Meeting (1), Pelletier (2), Round I (2), Wilson (1).

Tetrapedia Reinschiana Archer (10, p. 10, Fig. 6; 11, p. 118, Fig. 154).

Birch (0-1), Broughton (1), Clearwater (3), Crean (0-1), Fishing (0-1), Halkett (0-1), Kenderdine (0-2), Lenore (3), Madge (1), Midnight (1), Redberry (0-1), Stony (1), Turtle (0-1), Waskesiu (0-1).

OSCILLATORIACEAE

Spirulina major Kütz. (30, p. 50, Pl. 7, Fig. 1; 11, p. 347, Fig. 412).

Kenderdine (0-1), Last Mountain (0-2), Lenore (1), Little Quill (0-1), Manito (0-1), Margo (0-1), Murray (0-1).

Spirulina princeps W. and G. S. West (30, p. 50, Pl. 7, Fig. 2; 11, p. 348, Fig. 414).

Little Quill (0-1).

Oscillatoria limosa Ag. (11, p. 357, Fig. 420; 10, p. 212, Fig. 178).

Basin (1), Jackfish (0-2), Wilson (2).

Oscillatoria prolifica (Greville) Gomont (30, p. 51, Pl. 7, Figs. 3 to 5; 11, p. 369, Fig. 448).

Kingsmere (0-1), Last Mountain (0-1).

Oscillatoria tenuis Ag. (30, p. 52, Pl. 7, Fig. 6; 11, p. 362, Fig. 427, 428a).

Basin (1), Kenderdine (0-1), Little Quill (0-1), Margo (0-1), Wakaw (1), Waskesiu (0-1).

Trichodesmium lacustre Klebahn (30, p. 54, Pl. 8, Fig. 1; 11, p. 362, Fig. 436).

Meeting (1), Pelletier (1), Witchekan (1).

Lyngbya Birgei G. M. Smith (30, p. 54, Pl. 7, Figs. 14, 15; 11, p. 401, Fig. 505).

Bagwa (3), Birch (3), Broughton (2), Christopher (2), Crean (0-2), Echo (0-4), Edwards (1), Emma (1), Fishing (3), Greenwater (2), Halkett (2-4), Helene (2), Jackfish (1-2), Kingsmere (0-1), Last Mountain (0-2), Lavallee (1), Little Loon (4), Little Manitou (0-2), Madge (3), Margo (2-3), Meeting (3), Murray (0-3), Pasqua (1), Redberry (0-2), Round I (1), Round II (1), Shady (1), Stony (1), Sturgeon (2), Turtle (2-3), Wabeno (4), Wakaw (1), Waskesiu (0-1), Witchekan (1).

Lyngbya Birgei var. *minor* var. nov.

Atten (1), Basin (1), Big Quill (0-3), Last Mountain (0-1), Little Manitou (0-1), Little Quill (0-3), Margo (0-3), Murray (0-2), Sturgeon (2).

The specimens differ from the typical *L. Birgei* only in the smaller width of the cells. They measured 13 to 16.5 μ in diameter. This may perhaps be thought an effect of high salinity; but in several lakes the smaller forms were found together with filaments having cells 18 to 20 μ in diameter. Until further collections show that the minimal limits of *L. Birgei* should be lowered so as to include these smaller specimens, they are proposed as a new variety of this species.

Lyngbya Birgei var. *minor* var. nov. Differt a forma typica tantum dimensione cellulae. Cellulis 13-16.5 μ latis.

Lyngbya contorta Lemm. (30, p. 53, Pl. 7, Figs. 12, 13; 11, p. 397, Fig. 501).

Basin (2), Big Quill (3-4), Bitter (2), Good Spirit (1), Last Mountain (0-2), Midnight (1), Pasqua (1), Wilson (3).

Lyngbya limnetica Lemm. (30, p. 52, Pl. 7, Figs. 9 to 11; 11, p. 399, Fig. 504).

Broughton (2), Crean (0-4), Fishing (0-2), Good Spirit (3), Halkett (0-3), Helene (2), Jackfish (0-2), Kenderdine (0-2), Kingsmere (0-3), Margo (0-2), Midnight (2), Redberry (0-1), Shady (1), Stony (3), Turtle (0-2), Waskesiu (0-4), Witchekan (2).

Lyngbya putealis Mont. (11, p. 405, Fig. 517; 10, p. 193, Fig. 159).

Broughton (1). A single filament, probably introduced by migratory birds.

NOSTOCACEAE

Anabaena affinis Lemm. (30, p. 57, Pl. 8, Fig. 7; 11, p. 320, Fig. 374).

Kingsmere (0-2), Moose Creek (2), Waskesiu (0-1).

Anabaena circinalis (Kütz.) Rab. (30, p. 59, Pl. 9, Figs. 4, 5).

Bagwa (2), Crean (0-2), Halkett (0-2), Kingsmere (0-1), Last Mountain (0-2), Moose Creek (2), Waskesiu (0-3).

Anabaena flos-aquae (Lyngb.) Bréb. (30, p. 60, Pl. 10, Figs. 2 to 4; 11, p. 322, Fig. 379).

Bagwa (2), Birch (1-3), Brightsand (1-2), Christopher (2), Echo (0-3), Emma (2), Fishing (2-3), Greenwater (3-4), Halkett (0-3), Jackfish (0-3), Kenderdine (0-3), Kenossee (3), Kingsmere (0-3), Last Mountain (0-4), Lavallee (2), Little Loon (3), Madge (2), Margo (0-2), Meeting (2), Murray (2-5), Pasqua (2), Shady (3), Stony (1), Sturgeon (3), Turtle (1-2), Wabeno (3), Waskesiu (0-3), Wilson (2).

Anabaena Lemmermanni P. Richter (30, p. 61, Pl. 10, Fig. 8, Pl. 11, Fig. 1; 11, p. 322, Fig. 391).

Bagwa (2), Crean (0-1), Emma (2), Good Spirit (1), Greenwater (0-3), Halkett (2-4), Jackfish (0-2), Kingsmere (0-3), Last Mountain (0-3), Lavallee (2), Little Loon (2), Madge (2), Meeting (1), Moose Creek (4), Murray (0-3), Round I (3), Shady (2), Tibiska (3), Waskesiu (0-4), Waskesiu Narrows (2).

Frequently spore masses only were found.

Anabaena limnetica G. M. Smith (30, p. 57, Pl. 8, Fig. 8; 11, p. 316, Fig. 367).

Bagwa (2), Crean (0-1), Kingsmere (0-2), Lavallee (2), Shady (2), Tibiska (2), Waskesiu (0-2).

Anabaena macrospora var. *robusta* Lemm. (30, p. 57, Pl. 8, Fig. 6; 11, p. 322, Fig. 378). Waskesiu Narrows (3).

Anabaena spiroides Klebahn (11, p. 325; 10, p. 361, Fig. 296).

Bagwa (3), Greenwater (0-2), Halkett (0-1), Moose Creek (3), Pasqua (2), Round I (2), Shady (2), Tibiska (2), Waskesiu (0-3), Waskesiu Narrows (4).

To the Saskatchewan specimens apply the remarks of Moore and Carter (20), "The specimens were almost exactly similar to Klebahn's examples, the only difference being in the slightly smaller size of the spirals, which were about 30-40 μ in diameter, and the turns 30-40 μ apart, instead of 45-54 μ in the first case and 40-50 μ in the second as given by Klebahn".

Anabaena spiroides var. *crassa* Lemm. (30, p. 59, Pl. 9, Figs. 1 to 3; 11, p. 325, Fig. 383). Echo (0-2).

Nostoc Linckia (Roth) Born. (11, p. 298, Fig. 346; 10, p. 332, Fig. 276).

Waskesiu (0-1). In floating debris.

Aphanizomenon flos-aquae (L.) Ralfs (30, p. 61, Pl. 11, Figs. 2 to 4; 11, p. 290, Fig. 342).

Bagwa (3), Crean (0-1), Deep (2), Echo (0-4), Fishing (1), Greenwater (3), Kenossee (4), Kingsmere (0-2), Moose Creek (1), Murray (0-1), Pasqua (3), Round I (1), Shady (4), Sturgeon (3), Tibiska (2), Wakaw (3), Waskesiu (0-4), Waskesiu Narrows (4), Witchehan (1).

Nodularia spumigena Mertens (11, p. 289; 13, p. 200, Fig. 107).

Antelope (0-4), Fishing (0-1), Greenwater (0-1), Kenderdine (1-3), Little Quill (2-3), Redberry (0-3), Round I (1).

Moore (19) reported this species from Devil's Lake, North Dakota. Smith (32), commenting on this report, asserts, "Its occurrence as the most abundant and widely distributed of the Myxophyceae in the plankton of the lakes in North Dakota is most surprising, since not a single filament has been recorded from plankton surveys involving hundreds of lakes in other parts of the United States or in Great Britain". The Saskatchewan specimens were found only in saline lakes, the variety *major* being restricted to those with greater concentration of salts. That *N. spumigena* and its varieties float freely may be an effect of salinity and does not seem to be of rare occurrence. Lakowitz (16) records them as such from the coasts of Europe and North America and from saline lakes of continental Europe.

Nodularia spumigena var. *major* (Kütz.) Born. and Flah. (13, p. 201; 33, p. 185).

Antelope (0-3), Big Quill (0-1), Manito (1-4).

STIGONEMATACEAE

Stigonema hormoides (Kütz.) Born. and Flah. (11, p. 183, Fig. 219; 10, p. 397, Fig. 325).

Round II (1). Only fragments. Doubtful.

RIVULARIACEAE

Gloeotrichia echinulata (J. E. Smith) P. Richter (30, p. 63, Pl. 11, Figs. 5, 6; 11, p. 236, Fig. 285).

Carlyle (2), Edwards (1), Fishing (0-1), Good Spirit (1), Greenwater (3-4), Halkett (0-1), Kenossee (1), Lavallee (1), Madge (1), Margo (1-5), Meeting (1), Murray (0-1), Sturgeon (1).

CHLOROPHYCEAE

The green algae are represented by a greater number of species and varieties than any other phytoplankton group, though in actual quantity they never form the dominant member of the community. The commonest genera are *Dictyosphaerium*, *Pediastrum*, *Scenedesmus*, *Sphaerocystis*, *Staurastrum*, and *Tetraëdron*. The hardness of the water might account for the scarcity of the desmids. *Volvox mononae* was found only in Shady Lake as a common, and even abundant, constituent of a bloom. The green algae, as a whole, were more numerous and frequent in the freshwater lakes and those of low salinity. Most saline lakes have no outlet and their salinity is gradually increasing. Little Quill Lake, for instance, had a total solid content of 10,850 p.p.m. in 1920 (14). This had risen to 19,368 p.p.m. in 1938 (21), an increase of nearly 80% in 18 years. It is probable that most green algae are unable to adapt themselves to the increasing concentration of salts and disappear from the lakes. Several species of *Lagerheimia*, *Oocystis*, *Scenedesmus*, and *Sphaerocystis*, however, maintain themselves even in very high concentrations.

VOLVOACEAE

Pandorina morum Bory (30, p. 95, Pl. 16, Figs. 16, 17; 25, p. 427, Figs. 387 to 389; 26, p. 10, Pl. 2, Fig. 3).

Echo (0-1), Little Loon (1), Shady (1), Waskesiu (0-2).

Eudorina elegans Ehr. (30, p. 96, Pl. 19, Fig. 1; 25, p. 440, Figs. 394 to 401; 26, p. 9, Pl. 2, Fig. 2).

Greenwater (0-2).

Volvox mononae G. M. Smith (30, p. 99, Pl. 18, Fig. 1; 26, p. 12, Pl. 3, Fig. 2).

Shady (4).

PALMELLACEAE

Sphaerocystis Schroeteri Chod. (30, p. 101, Pl. 19, Figs. 3, 4).

Antelope (0-2), Atten (1), Beartrap Creek (1), Birch (0-2), Christopher (3), Clearwater (1), Crean (0-1), Echo (0-2), Emma (1), Greenwater (0-2), Halkett (1-3), Jackfish (0-2), Kenderdine (0-2), Kingsmere (0-2), Last Mountain (0-2), Little Loon (1), Margo (0-2), Meeting (1), Murray (0-2), Round I (2), Shady (1), Tibiska (1), Turtle (1), Wabeno (1), Waskesiu (0-3).

Gloeocystis gigas (Kütz.) Lagerh. (30, p. 101, Pl. 19, Fig. 2).

Brightsand (0-1), Crean (0-1), Little Quill (0-2), Pelletier (1), Waskesiu (0-1).

Asterococcus limneticus G. M. Smith (30, p. 104, Pl. 20, Figs. 7 to 10).

Halkett (0-1).

CHLORANGIACEAE

Stylosphaeridium stipitatum (Bachm.) Geitler and Gimesi (32, p. 364, Fig. 244; 25, p. 482, Figs. 437, 438). Found only on *Coelosphaerium Naegelsanum*.

Antelope (0-1), Birch (0-1), Margo (0-1), Shady (1), Tibiska (1), Waskesiu (0-1).

COCOCOMYXACEAE

Elaktothrix gelatinosa Wille (30, p. 139, Pl. 34, Figs. 1 to 3; 8, p. 41, Pl. 2, Fig. 14).

Birch (0-1), Kenderdine (0-1).

ULOTRICHACEAE

Ulothrix subconstricta G. S. West (30, p. 179, Pl. 50, Figs. 4, 5).

Christopher (1), Greenwater (0-2), Pasqua (1), Redberry (0-1).

Ulothrix zonata (Web. and Mohr) Kütz. (30, p. 179, Pl. 50, Fig. 6; 7, p. 185, Pl. 6, Fig. 64).

Kingsmere (0-1).

CLADOPHORACEAE

Cladophora crispata (Roth) Kütz. (12, p. 40, Fig. 40; 7, p. 354).

Basin (2), Big Quill (0-2).

Cladophora fracta (Dillw.) Kütz. (12, p. 42, Figs. 41 to 44; 7, p. 353).

Beartrap Creek (3), Echo (0-1), Last Mountain (0-2).

Cladophora glomerata (L.) Kütz. (12, p. 35, Figs. 14, 15, 39; 7, p. 351, as *C. canalicularis*).

Little Manitou (0-3), Manito (0-2).

OEDOGONIACEAE

Oedogonium sp.

Little Manitou (1-4). Specimens were found in all collections and, at times, abundantly; all filaments, however, were sterile. The high salinity, about three times that of the ocean, probably causes the form to reproduce only vegetatively. Cells were 12 to 30 μ in width, 45 to 105 μ in length.

ULVACEAE

Enteromorpha prolifera var. **tubulosa** (Kütz.) Reinbold (7, p. 203).

Basin (1), Little Manitou (0-3), Manito (0-3).

CHLOROCOCCACEAE

Golenkinia paucispina W. and G. S. West (30, p. 127, Pl. 29, Figs. 4, 5).

Last Mountain (0-1).

Golenkinia radiata Chod. (30, p. 127, Pl. 29, Figs. 2, 3).

Clearwater (1), Last Mountain (0-1).

CHARACIACEAE

Characium gracilipes Lambert (30, p. 178, Pl. 49, Figs. 20 to 24; 7, p. 151, Pl. 5, Fig. 36).

Kingsmere (0-1).

HYDRODICTYACEAE

Pediastrum biradiatum Meyen (30, p. 173, Pl. 48, Figs. 5 to 8; 7, p. 179).

Waskesiu (0-1).

Pediastrum Boryanum (Turp.) Menegh. (30, p. 169, Pl. 46, Figs. 2 to 7; 7, p. 177).

Antelope (0-1), Atten (1), Basin (2), Beartrap Creek (1), Big Quill (0-2), Birch (3), Brightsand (2), Broughton (2), Christopher (2), Crean (0-2), Deep (1), Echo (0-2), Emma (3), Fishing (0-3), Good Spirit (1), Greenwater (0-2), Halkett (1-2), Helene (3), Jackfish (1-3), Kenderdine (0-3), Last Mountain (1-3), Lavallee (2), Lenore (1), Little Loon (3), Little Manitou (0-1), Little Quill (1-3), Manito (0-1), Margo (0-3), Meeting (3), Midnight (3), Moose Creek (1), Pasqua (2), Pelletier (1), Redberry (0-2), Round II (2), Sandy Beach (1), Shady (1), Soda (0-2), Stony (3), Sturgeon (2), Tibiska (1), Turtle (2-3), Wabeno (2), Wakaw (1), Waskesiu (0-3), Wilson (1), Witchekan (2).

Pediastrum Boryanum var. **undulatum** Wille (30, p. 170, Pl. 46, Fig. 8; 7, p. 178).

Waskesiu (0-1).

Pediastrum duplex Meyen (30, p. 171, Pl. 46, Figs. 14 to 16; 7, p. 179).

Big Quill (0-1), Birch (0-1), Broughton (1), Carlyle (1), Christopher (1), Crean (0-1), Echo (0-2), Helene (2), Jackfish (0-2), Kenderdine (0-1), Kingsmere (0-1), Last Mountain (0-3), Little Manitou (0-1), Margo (0-2), Meeting (2), Moose Creek (1), Murray (0-1), Pasqua (1), Redberry (0-1), Round I (1), Stony (1), Turtle (2-3), Waskesiu (0-2).

Pediastrum duplex var. **clathratum** (A. Br.) Lagerh. (30, p. 171, Pl. 47, Figs. 1 to 3; 7, p. 179).

Broughton (2), Echo (0-2), Helene (2), Kenderdine (0-1), Kingsmere (0-1), Last Mountain (0-2), Lavallee (1), Margo (0-1), Meeting (2), Murray (0-2), Redberry (0-1), Waskesiu (0-2).

Pediastrum duplex var. **gracillimum** W. and G. S. West (30, p. 172, Pl. 47, Figs. 8 to 11; 8, p. 54).

Broughton (1), Jackfish (0-1), Last Mountain (0-2), Murray (0-1), Waskesiu (0-1), Witchekan (2).

Pediastrum integrum Näg. (30, p. 168, Pl. 45, Fig. 7; 8, p. 54).

Christopher (1), Kingsmere (0-1), Little Quill (1), Waskesiu (0-1).

Pediastrum Kawraatskyl Schmidle (30, p. 170, Pl. 46, Figs. 10 to 13; 8, p. 54).

Brightsand (0-3), Helene (2), Meeting (2), Redberry (0-1), Stony (3), Turtle (0-1), Waskesiu (0-1), Witchekan (3).

Pediastrum simplex (Meyen) Lemm. (27, p. 56, Pl. 10, Figs. 10, 11; 7, p. 177).

Waskesiu (0-1).

Pediastrum simplex var. **duodenarium** (Bailey) Rab. (30, p. 167, Pl. 45, Figs. 2 to 6).

Birch (0-1), Redberry (0-1), Waskesiu (0-1), Witchekan (1).

Pediastrum tetras (Ehr.) Ralfs (30, p. 173, Pl. 48, Figs. 9 to 12; 7, p. 179).

Midnight (1), Stony (1), Turtle (0-1).

Sorastrum americanum var. **undulatum** G. M. Smith (30, p. 163, Pl. 44, Figs. 2, 3).

Good Spirit (1), Lavallee (1), Waskesiu (0-1).

Sorastrum spinulosum Näg. (30, p. 163, Pl. 44, Figs. 4, 5).

Big Quill (0-1), Good Spirit (1), Halkett (0-1), Jackfish (0-1), Last Mountain (0-1), Little Quill (0-2), Midnight (2), Redberry (0-2), Waskesiu (0-1).

COELASTRACEAE

Coelastrum microporum Näg. (30, p. 160, Pl. 41, Figs. 12, 13, Pl. 42, Fig. 1).

Helene (1), Last Mountain (0-1), Murray (0-1), Round I (2), Wabeno (1), Waskesiu (0-2).

OOCYSTACEAE

Chlorella vulgaris Beyerinck (30, p. 108, Pl. 22, Fig. 1).

Jackfish (0-1).

Westella botryoides (W. West) de Wildm. (30, p. 107, Pl. 21, Fig. 4; 8, p. 52, Pl. 2, Fig. 17).

Jackfish (0-2), Lenore (2), Madge (1), Waskesiu (0-1).

Dictyosphaerium pulchellum Wood (30, p. 105, Pl. 20, Fig. 13, Pl. 21, Fig. 1).

Birch (1), Crean (0-1), Emma (1), Halkett (0-1), Jackfish (0-2), Kenderdine (1), Kingsmere (0-1), Last Mountain (0-2), Lavallee (3), Madge (1), Meeting (1), Moose Creek (1), Murray (0-1), Pasqua (1), Pelletier (2), Stony (2), Tibiska (1), Turtle (0-2), Waskesiu (0-3), Wilson (1), Witchekan (1).

Trochiscia reticularis (Reinsch) Hansg. (30, p. 109, Pl. 22, Fig. 2; 7, p. 145, Pl. 4, Fig. 32).

Good Spirit (1), Halkett (0-1).

Planktosphaeria gelatinosa G. M. Smith (30, p. 103, Pl. 20, Figs. 3 to 6).

Waskesiu (0-2).

Oocystis Borgei Snow (30, p. 111, Pl. 22, Fig. 4).

Antelope (0-1), Birch (2-3), Echo (0-1), Fishing (0-1), Halkett (0-1), Last Mountain (0-2), Margo (2-3), Redberry (0-2), Tibiska (1), Turtle (0-1), Waskesiu (0-1).

Oocystis crassa Wittr. (30, p. 113, Pl. 22, Figs. 12, 13).

Jackfish (0-2), Last Mountain (0-1), Lenore (1), Manito (0-3), Margo (0-1).

Oocystis elliptica W. West (30, p. 111, Pl. 22, Fig. 5).

Last Mountain (0-2), Stony (2).

Oocystis eremosphaeria G. M. Smith (30, p. 113, Pl. 23, Figs. 1, 2).

Echo (0-2).

Oocystis lacustris Chod. (30, p. 112, Pl. 22, Figs. 8, 9).

Antelope (0-1), Birch (2-3), Echo (0-2), Helene (2), Jackfish (0-1), Kenderdine (0-2), Kenosee (1), Lenore (3), Little Quill (0-2), Madge (1), Margo (0-2), Meeting (1), Murray (0-1), Pasqua (1), Stony (1), Waskesiu (0-1), Wilson (1).

Oocystis parva W. and G. S. West (30, p. 112, Pl. 22, Fig. 3).

Helene (2), Meeting (1), Murray (0-1), Waskesiu (0-1).

Oocystis pusilla Hansg. (30, p. 111, Pl. 22, Fig. 3).

Kenderdine (0-3), Waskesiu (0-1), Witchekan (2).

Oocystis solitaria Wittr. (30, p. 113, Pl. 22, Fig. 11).

Echo (0-2), Jackfish (0-2).

Lagerheimia ciliata (Lagerh.) Chod. (30, p. 129, Pl. 31, Figs. 1, 2).

Pelletier (2), Waskesiu (0-1).

Lagerheimia Droescheri (Lemm.) Printz (30, p. 131, Pl. 30, Figs. 5 to 7).

Basin (1), Fishing (0-1), Kenderdine (0-1).

Lagerheimia longiseta (Lemm.) Printz (30, p. 130, Pl. 30, Figs. 8, 9).

Redberry (0-1).

Lagerheimia subsalsa Lemm. (30, p. 130, Pl. 30, Figs. 3, 4).

Basin (1), Clearwater (1), Kenderdine (0-1).

Dimorphococcus lunatus A. Br. (30, p. 106, Pl. 21, Fig. 5).

Jackfish (0-1), Lenore (1), Pelletier (1), Redberry (0-1).

Ankistrodesmus spiralis (Turner) Lemm. (30, p. 135, Pl. 32, Figs. 6, 7).

Clearwater (1), Halkett (0-1), Jackfish (0-1), Kenderdine (0-1), Last Mountain (0-1), Moose Creek (1).

Selenastrum gracile Reinsch (30, p. 133, Pl. 31, Fig. 5).

Waskesiu (0-2).

Selenastrum Westli G. M. Smith (30, p. 133, Pl. 31, Figs. 8 to 10).

Brightsand (0-2), Witchekan (1).

Kirchneriella lunaris var. **Dianae** Bohl. (30, p. 141, Pl. 34, Fig. 5).

Waskesiu (0-1).

Kirchneriella obesa (W. West) Schmidle (30, p. 142, Pl. 35, Figs. 2, 3).

Manito (0-2), Stony (2), Waskesiu (0-1).

Tetraëdron caudatum (Corda) Hansg. (30, p. 120, Pl. 25, Figs. 4 to 7).

Kenderdine (0-1), Midnight (1), Turtle (0-1), Witchekan (1).

Tetraëdron hastatum var. **palatinum** (Schmidle) Lemm. (30, p. 121, Pl. 25, Figs. 19 to 21).

Waskesiu (0-1).

Tetraëdron limneticum Borge (30, p. 123, Pl. 27, Figs. 1 to 3).

Waskesiu (0-2).

Tetraëdron trigonum var. **gracile** (Reinsch) de Toni (30, p. 117, Pl. 24, Figs. 5 to 9).

Waskesiu (0-1).

Tetraëdron trigonum var. **minor** Reinsch (27, p. 68, Pl. 14, Figs. 39, 40).

Clearwater (3), Fishing (0-1), Lenore (2).

SCENEDESMACEAE

Scenedesmus arcuatus Lemm. (30, p. 153, Pl. 38, Figs. 12 to 14).

Birch (0-2), Brightsand (0-1), Fishing (0-1), Helene (2), Kenderdine (0-1), Meeting (1), Midnight (2), Turtle (0-1).

Scenedesmus bijuga (Turp.) Lagerh. (30, p. 152, Pl. 37, Figs. 18 to 20).

Brightsand (0-1), Christopher (1), Clearwater (2), Halkett (0-1), Helene (3), Jackfish (0-1), Kenderdine (0-3), Last Mountain (0-1), Lenore (2), Margo (0-1), Meeting (1), Midnight (2), Pasqua (1), Pelletier (1), Stony (2), Tibiska (1), Waskesiu (0-1), Wilson (2), Witchekan (2).

Scenedesmus bijuga var. **alternans** (Reinsch) Borge (30, p. 153, Pl. 38, Figs. 10, 11).

Kenderdine (0-1), Lenore (1).

Scenedesmus dimorphus (Turp.) Kütz. (30, p. 151, Pl. 37, Figs. 15 to 17).

Kenderdine (0-1).

Scenedesmus obliquus (Turp.) Kütz. (30 p. 151, Pl. 37, Figs. 12 to 14).

Fishing (0-1), Wakaw (1), Witchekan (2).

Scenedesmus quadricauda (Turp.) Bréb. (30, p. 158, Pl. 40, Figs. 9 to 11).

Basin (1), Birch (1), Broughton (2), Christopher (2), Clearwater (1), Echo (0-1), Fishing (0-3), Halkett (0-1), Helene (3), Kenderdine (0-2), Last Mountain (0-1), Lenore (2), Margo (0-2), Meeting (1), Midnight (1), Murray (0-1), Round II (1), Stoney (1), Stony (2), Turtle (2), Waskesiu (0-1).

Scenedesmus quadricauda var. **parvus** G. M. Smith (30, p. 158, Pl. 40, Fig. 17).

Broughton (2).

Scenedesmus quadricauda var. **quadrispina** (Chod.) G. M. Smith (30, p. 158, Pl. 40, Figs. 15, 16).

Lenore (2), Midnight (1), Redberry (0-1), Wilson (1).

Crucigenia irregularis Wille (30, p. 145, Pl. 36, Figs. 4, 5).

Halkett (0-2), Helene (1), Moose Creek (1), Waskesiu (0-1).

Crucigenia quadrata Morren (30, p. 147, Pl. 36, Figs. 10 to 14).

Fishing (0-1), Jackfish (0-1), Little Quill (0-3), Madge (1), Witchekan (0-3).

Crucigenia rectangularis (Näg.) Gay (30, p. 144, Pl. 36, Fig. 3).

Christopher (3), Stony (1).

Actinastrum gracillimum G. M. Smith (30, p. 164, Pl. 43, Figs. 3 to 5).

Waskesiu (0-1).

DESMIDIACEAE

Closterium acerosum (Schränk) Ehr. (34, Vol. 1, p. 146, Pl. 18, Figs. 2 to 5; 31, p. 10, Pl. 53, Fig. 1).

Echo (0-1), Jackfish (0-1), Last Mountain (0-2), Margo (1), Meeting (1), Murray (0-1), Wabeno (1).

Closterium acerosum var. **elongatum** Bréb. (34, Vol. 1, p. 147, Pl. 18, Fig. 1).

Kenderdine (0-1), Pasqua (1).

Closterium aciculare T. West (31, p. 11).

Round II (3).

Closterium aciculare var. **subprorum** W. and G. S. West (34, Vol. 1, p. 175, Pl. 23, Figs. 4, 5; 31, p. 11, Pl. 53, Fig. 3).

Halkett (0-3), Jackfish (0-1), Murray (0-1), Round II (2), Soda (0-1), Waskesiu (0-1).

Closterium acutum (Lyngb.) Bréb. (34, Vol. 1, p. 177, Pl. 23, Figs. 9 to 14; 31, p. 11, Pl. 53, Fig. 4).

Halkett (0-1).

Closterium gracile var. **elongatum** W. and G. S. West. (34, Vol. 1, p. 168, Pl. 21, Figs. 14 to 16; 31, p. 11, Pl. 53, Fig. 2).

Sturgeon (1), Waskesiu (0-1).

Closterium moniliferum (Bory) Ehr. (34, Vol. 1, p. 142, Pl. 16, Figs. 15, 16; 31, p. 9, Pl. 52, Fig. 10).

Beartrap Creek (1), Deep (1), Echo (0-1), Jackfish (0-1).

Closterium parvulum Næg. (34, Vol. 1, p. 133, Pl. 15, Figs. 9 to 12).

Kenderdine (0-2).

Closterium prorum Bréb. (34, Vol. 1, p. 173, Pl. 23, Figs. 1 to 3).

Edwards (1), Kenderdine (0-1).

Closterium Venus Kütz. (34, Vol. 1, p. 137, Pl. 15, Figs. 15 to 20; 31, p. 9, Pl. 52, Fig. 9).

Kingmere (0-1).

Pleurotaenium coronatum (Bréb.) Rab. (34, Vol. 1, p. 199, Pl. 27, Figs. 16 to 18, Pl. 28, Fig. 4).

Waskesiu (0-1). Doubtful.

- Pleurotaenium maximum** (Reinsch) Lund. (34, Vol. 1, p. 213, Pl. 31, Figs. 1, 2).
Kingsmere (0-1).
- Cosmarium asphaerosporum** Nordst. (34, Vol. 2, p. 163, Pl. 60, Figs. 24, 25).
Clearwater (1).
- Cosmarium cyclicum** var. **Nordstedtianum** (Reinsch) W. and G. S. West (34, Vol. 2, p. 146, Pl. 58, Fig. 12; 31, p. 32, Pl. 57, Figs. 19, 20).
Fishing (0-2), Last Mountain (0-1), Meeting (2), Pasqua (1), Turtle (0-), Waskesiu (0-1).
- Cosmarium depressum** var. **achondrum** (Boldt) W. and G. S. West (34, Vol. 2, p. 177, Pl. 62, Figs. 6 to 9; 31, p. 30, Pl. 57, Figs. 5, 6).
Halkett (0-1), Waskesiu (0-1).
- Cosmarium granatum** Bréb. (34, Vol. 2, p. 186, Pl. 63, Figs. 1 to 3).
Lenore (3).
- Cosmarium laeve** var. **septentrionale** Wille (34, Vol. 3, p. 102, Pl. 73, Figs. 22 to 25).
Turtle (0-1).
- Cosmarium margaritiferrum** Menegh. (34, Vol. 3, p. 199, Pl. 83, Figs. 4 to 11).
Echo (0-1), Kenderdine (0-1).
- Cosmarium Meneghinii** Bréb. (34, Vol. 3, p. 90, Pl. 72, Figs. 29 to 32).
Clearwater (1), Kenderdine (0-1), Madge (2), Soda (0-1), Turtle (1).
- Cosmarium punctulatum** var. **subpunctulatum** (Nordst.) Börg. (34, Vol. 3, p. 209, Pl. 84, Figs. 15 to 20).
Kenderdine (0-1).
- Cosmarium reniforme** (Ralfs) Archer (34, Vol. 3, p. 157, Pl. 79, Figs. 1, 2, Pl. 82, Fig. 15; 31, p. 33, Pl. 57, Fig. 23).
Echo (0-1).
- Cosmarium subtumidum** var. **Klebsii** (Gutw.) W. and G. S. West (34, Vol. 2, p. 193, Pl. 63, Figs. 21 to 23).
Halkett (0-1), Tibiska (1), Turtle (0-1), Wabeno (1).
- Cosmarium tenue** Archer (34, Vol. 2, p. 167, Pl. 61, Figs. 12 to 15).
Kenderdine (0-1), Lenore (2), Turtle (0-1), Wilson (1), Witchehan (1).
- Staurostrum anatinum** Cooke and Wills (34, Vol. 5, p. 142, Pl. 146, Fig. 7, Pl. 147, Fig. 1).
Greenwater (0-1), Halkett (0-2), Moose Creek (1), Stony (1), Turtle (0-1), Waskesiu (0-2).
- Staurostrum Chaetoceras** (Schröd.) G. M. Smith (31, p. 99, Pl. 76, Figs. 21 to 24, Pl. 77, Fig. 1).
Waskesiu (0-1).
- Staurostrum contortum** G. M. Smith (31, p. 98, Pl. 76, Figs. 17 to 20).
Helene (1), Stony (1), Waskesiu (0-1).
- Staurostrum curvatum** W. West (34, Vol. 5, p. 19, Pl. 130, Figs. 15, 16; 31, p. 73, Pl. 69, Figs. 4 to 9).
Helene (1), Waskesiu (0-1).
- Staurostrum cuspidatum** Bréb. (34, Vol. 5, p. 23, Pl. 132, Figs. 13 to 15; 31, p. 74, Pl. 68, Figs. 27 to 34).
Waskesiu (0-1).
- Staurostrum gracile** Ralfs (34, Vol. 5, p. 96, Pl. 144, Figs. 3 to 7; 31, p. 88, Pl. 73, Figs. 16 to 18).
Crean (1), Greenwater (0-1), Halkett (0-2), Kenderdine (0-2), Kingsmere (0-1), Meeting (2), Murray (0-1), Waskesiu (0-1).
- Staurostrum leptocladum** Nordst. (31, p. 102, Pl. 78, Figs. 1 to 7).
Beartrap Creek (1), Echo (0-1), Kingsmere (0-1), Lavallee (2).

Staurastrum leptocladum var. **sinuatum** Wolle (31, p. 104, Pl. 78, Figs. 12 to 14).

Antelope (0-1).

Staurastrum longiradiatum W. and G. S. West (31, p. 90, Pl. 74, Figs. 5 to 11).

Birch (2), Broughton (1), Crean (0-2), Emma (3), Greenwater (0-2), Halkett (2-3), Jackfish (0-2), Kenderdine (0-2), Kingsmere (0-2), Margo (0-1), Meeting (3), Midnight (1), Murray (0-3), Soda (0-2), Wabeno (2), Waskesiu (0-2), Witchehan (1).

Staurastrum megacanthum Lund. (34, Vol. 5, p. 20, Pl. 131, Figs. 7, 8; 31, p. 75, Pl. 69, Figs. 16 to 21).

Turtle (0-1).

Staurastrum paradoxum Meyen (34, Vol. 5, p. 101, Pl. 145, Figs. 1 to 5; 31, p. 85, Pl. 72, Figs. 15 to 22, Pl. 73, Figs. 1, 2).

Basin (1), Big Quill (0-2), Brightsand (1-2), Broughton (2), Crean (0-2), Deep (1), Echo (0-1), Emma (2), Fishing (0-1), Greenwater (0-3), Helene (2), Jackfish (0-2), Kenderdine (0-3), Last Mountain (0-1), Margo (0-2), Meeting (3), Moose Creek (1), Pasqua (3), Pelletier (1), Redberry (0-1), Round II (1), Sandy Beach (1), Shady (1), Soda (0-1), Stony (2), Sturgeon (1), Turtle (0-2), Wabeno (2), Waskesiu (0-2).

Staurastrum paradoxum var. **longipes** Nordst. (34, Vol. 5, p. 103, Pl. 146, Figs. 2, 3; 31, p. 86, Pl. 73, Figs. 3 to 6).

Waskesiu (0-2).

Staurastrum protectum W. and G. S. West (31, p. 87).

Waskesiu (0-1).

Arthrodesmus incus (Bréb.) Hass. (34, Vol. 4, p. 90, Pl. 113, Figs. 13 to 15; 31, p. 131, Pl. 85, Figs. 19 to 22).

Waskesiu (0-1).

Spondylosium moniliforme Lund. (31, p. 141, Pl. 87, Figs. 5 to 7).

Waskesiu (0-1). Doubtful.

Hyalotheca dissiliens (Smith) Bréb. (31, p. 142, Pl. 87, Figs. 8, 9).

Last Mountain (0-1), Turtle (0-1).

THE PHYTOPLANKTON OF SOUTHERN AND CENTRAL SASKATCHEWAN

PART II¹

By PAUL E. KUEHNE

BACILLARIEAE

Asterionella, *Fragilaria*, *Melosira*, *Stephanodiscus*, and *Tabellaria*, being true plankton diatoms and cosmopolitan in their distribution, are found in almost all freshwater and many of the saline lakes. At times they are abundant. Other genera, such as *Navicula*, *Pinnularia*, *Nitzschia*, *Gyrosigma*, *Cymatopleura*, *Rhopalodia*, etc., are not so widely distributed in the Saskatchewan lakes and usually only a few individuals can be found, many of them only in the littoral collections. They are really representatives of the *benthos* or shore and bottom diatoms and have drifted into the plankton through the action of waves or the circulating water. Most of them are represented in both saline and freshwater lakes. *Pleurosigma elongatum*, *Tropidoneis lepidoptera*, *Amphiprora alata*, *Campylodiscus clypeus*, *Rhoicosphenia curvata*, and *Surirella Baileyana* occur only in the saline lakes. Several other species of *Surirella* also occur mostly in the saline lakes but the species *S. biseriata*, *elegans*, and *spiralis*, and the two *Gyrosigma* species were only found in freshwater. Most diatoms are essentially cold water organisms and should, therefore, prove more common during the early spring and late fall. The importance of temperature effect on diatom occurrence may be gathered from the fact that Bailey (1) reported 63 species, mostly shore and bottom diatoms, from the Quill lakes. His collections were made during May.

COSCINODISCEAE

Melosira crenulata (Ehr.) Kütz. (5, p. 29).

Crean (0-3), Kingsmere (0-3), Moose Creek (1), Stony (2), Waskesiu (0-3).

Melosira granulata (Ehr.) Ralfs (15, p. 87, Fig. 44; 5, p. 30).

Antelope (0-1), Beartrap Creek (1), Birch (1), Broughton (3), Crean (1-4), Echo (0-3), Emma (3), Good Spirit (1), Greenwater (2), Halkett (2-3), Helene (2), Jackfish (0-2), Kingsmere (0-3), Last Mountain (0-1), Lavallee (4), Little Loon (3), Madge (2), Margo (0-1), Meeting (3), Murray (2-5), Pasqua (3), Round I (3), Shady (2), Stony (2), St. Tibiska (3), Turtle (3), Wabeno (2), Wakaw (1), Waskesiu (1-5), Witchehan (1).

Melosira varians Ag. (15, p. 103, Fig. 69; 5, p. 40).

Broughton (1), Christopher (1), Crean (0-2), Last Mountain (0-1), Stony (2), Sturgeon (1), Waskesiu (0-2).

Cyclotella comta (Ehr.) Kütz. (15, p. 103, Fig. 69; 5, p. 40).

Brightsand (0-2), Echo (0-2), Emma (2), Fishing (0-1), Halkett (0-2), Jackfish (0-1), Kenderdine (0-1), Kingsmere (0-2), Little Quill (0-2), Madge (1), Tibiska (1), Turtle (2), Wabeno (1), Waskesiu (0-2).

¹ Part I appeared in the August issue.

Cyclotella Meneghiniana Kütz. (15, p. 100, Fig. 67; 5, p. 38).

Antelope (0-2), Bitter (3), Brightsand (2), Deep (3), Echo (0-3), Emma (2), Fishing (0-1), Last Mountain (0-2), Lenore (2), Little Loon (2), Little Manitou (0-3), Redberry (0-2).

Cyclotella operculata (Ag.) Kütz. (15, p. 102, Fig. 66; 5, p. 39).

Brightsand (0-2), Deep (3), Echo (0-4), Emma (2), Fishing (0-1), Jackfish (0-1), Kenderdine (0-1), Pelletier (1), Turtle (0-2).

Cyclotella quillensis Bailey (5, p. 38).

Little Quill (0-1).

Stephanodiscus niagarae Ehr. (5, p. 61).

Antelope (0-2), Bagwa (1), Birch (2), Bitter (1), Brightsand (2), Broughton (2), Carlyle (1), Christopher (1), Crean (1-3), Deep (3), Echo (0-4), Emma (3), Fishing (1-3), Good Spirit (1), Greenwater (2), Halkett (1-3), Helene (2), Jackfish (0-2), Kenderdine (1-3), Kenosee (3), Kingsmere (0-3), Last Mountain (0-3), Lavallee (3), Little Loon (2), Little Quill (1-2), Margo (0-2), Meeting (3), Murray (2-3), Pasqua (3), Soda (1), Sturgeon (2), Tibiska (3), Turtle (3), Wabeno (2), Wakaw (2), Waskesiu (1-4), Waskesiu Narrows (1), Witcheakan (1).

CHAETOCERACEAE

Chaetoceros Elmorei Boyer (5, p. 112).

Antelope (3-5), Big Quill (0-2), Bitter (5), Carlyle (1), Fishing (1-2), Good Spirit (1), Jackfish (0-2), Last Mountain (0-1), Lenore (1), Little Quill (3-4), Madge (1), Redberry (1-4), Stoney (2), Wilson (1).

The genus *Chaetoceros* was considered wholly marine, though investigators had described forms from several European saline lakes (15). The first American specimens were reported from Devil's Lake, North Dakota, under the name *C. Elmorei* (4). The discovery aroused considerable interest, because this lake is so very far inland. Seven years later, Bailey (1) found abundant specimens in the collections from Little Quill Lake. Though he admitted that most of them "closely resembled the form described and figured by C. S. Boyer", he established a new species, *C. quillensis*. In a later publication (5), Boyer writes, "After many examinations of the material from Little Quill Lake, Saskatchewan, I am unable to distinguish the forms of *Chaetoceros* from the species found in Devil's Lake, North Dakota, although the Quill Lake material contains more of the immature and shorter filaments, the results of colder habitat". The recent collections contained much material and furnish proof that Boyer's conclusions must be accepted as correct, and that the forms from Saskatchewan and North Dakota belong to the same species. Not only were many found with vegetative structures as described and figured by Boyer, but all agreed in the shape and formation of the reproductive body. The variations in the vegetative structures, such as size of cells and divergence of setae, may, perhaps, be considered seasonal changes, similar to those found in other algae, e.g., *Ceratium hirundinella*. It may be added that the lakes of North Dakota and Saskatchewan are the only ones on this continent from which *Chaetoceros* is reported.

TABELLARIACEAE

Tabellaria fenestrata (Lyngb.) Kütz. (15, p. 122, Fig. 99; 5, p. 151).

Atten (2), Brightsand (2), Crean (2-4), Deep (3), Echo (0-1), Good Spirit (1), Halkett (0-3), Kingsmere (0-3), Last Mountain (0-3), Lavallee (2), Moose Creek (1), Pasqua (2), Redberry (1-3), Round II (2), Shady (3), Tibiska (4), Turtle (0-2), Wabeno (3), Wakaw (3), Waskesiu (0-2).

MERIDIONACEAE

Meridion circulare var. **constricta** (Ralfs) Van Heurck (15, p. 131, Fig. 119; 5, p. 172).

Murray (0-1). In floating debris.

FRAGILARIACEAE

Fragilaria capucina Desmaz. (15, p. 138, Fig. 126; 5, p. 187).

Big Quill (0-2), Bitter (2), Christopher (1), Crean (0-1), Deep (2), Echo (3), Emma (2), Fishing (0-2), Greenwater (2), Halkett (1-2), Helene (2), Jackfish (1-3), Kenderdine (0-1), Kingsmere (0-2), Last Mountain (0-4), Lavallee (2), Lenore (2), Murray (0-2), Round I (1), Round II (1), Sturgeon (1), Tibiska (2), Wabeno (1), Wakaw (2), Waskesiu (0-3).

Fragilaria crotonensis Kitton (15, p. 137, Fig. 125; 5, p. 187).

Atten (1), Basin (2), Big Quill (0-2), Birch (3), Bitter (3), Brightsand (3), Broughton (2), Carlyle (1), Christopher (2), Crean (2-4), Deep (3), Echo (1-5), Edwards (1), Emma (3), Fishing (1-3), Good Spirit (1), Greenwater (3), Halkett (2-4), Helene (2), Jackfish (1-3), Kenderdine (2-3), Kenosee (3), Kingsmere (1-3), Last Mountain (0-4), Lavallee (4), Little

Loon (3), Madge (2), Manito (0-1), Margo (0-2), Meeting (3), Midnight (2), Moose Creek (1), Murray (2-4), Pasqua (3), Pelletier (1), Redberry (0-1), Round I (3), Round II (3), Shady (3), Soda (0-1), Stony (2), Sturgeon (2), Tibiska (3), Turtle (3), Wabeno (3), Wakaw (3), Waskesiu (0-4).

Fragilaria virescens Ralfs (15, p. 142, Fig. 144; 5, p. 184).

Helene (2), Midnight (2), Stony (2).

Synedra acus Kütz. (15, p. 155, Fig. 170; 5, p. 201).

Brightsand (2-3), Echo (0-2), Fishing (0-2), Helene (1), Jackfish (0-2).

Synedra acus var. **angustissima** Grunow (15, p. 155, Fig. 172).

Brightsand (0-3), Deep (3).

Synedra acus var. **radians** (Kütz.) Hust. (15, p. 155, Fig. 171; 5, p. 202).

Brightsand (0-1), Deep (3), Echo (0-2), Helene (2), Kenderdine (0-2), Last Mountain (0-3), Madge (1), Meeting (3), Pelletier (1), Turtle (3).

Synedra pulchella (Ralfs) Kütz. (15, p. 160, Fig. 187).

Wakaw (2).

Synedra ulna (Nitzsch) Ehr. (15, p. 151, Figs. 158, 159; 5, p. 198).

Atten (1), Beartrap Creek (1), Birch (0-2), Bitter (2), Brightsand (2-3), Christopher (1), Clearwater (1), Crean (0-2), Deep (2), Echo (0-2), Emma (2), Fishing (0-3), Halkett (1-2), Helene (2), Jackfish (0-3), Kenderdine (0-1), Last Mountain (0-2), Margo (0-1), Murray (1-2), Pasqua (2), Pelletier (1), Round II (2), Sandy Beach (1), Turtle (1-2), Wabeno (1), Waskesiu (0-1), Witchehan (2).

Synedra ulna var. **biceps** (Kütz.) Hust. (15, p. 154, Fig. 166).

Bitter (3), Brightsand (2-3), Deep (3), Jackfish (0-3), Sturgeon (1), Wakaw (1).

Synedra ulna var. **danica** (Kütz.) Grunow (15, p. 154, Fig. 168; 5, p. 200).

Deep (3), Wakaw (2).

Synedra ulna var. **Ramesi** (Heri. and Perag.) Hust. (15, p. 152, Fig. 163).

Fishing (1).

Asterionella formosa Hass. (15, p. 147, Fig. 156; 5, p. 213).

Basin (1), Birch (2-3), Bitter (2), Brightsand (2), Carlyle (2), Christopher (3), Clearwater (1), Crean (0-3), Deep (2), Echo (0-2), Emma (3), Greenwater (0-1), Halkett (0-2), Helene (2), Jackfish (1-4), Kenderdine (0-3), Kenosee (3), Kingsmere (0-3), Last Mountain (0-3), Lavallee (2), Lenore (1), Little Loon (3), Madge (1), Meeting (2), Murray (3-4), Pelletier (1), Redberry (0-2), Round I (4), Round II (2), Sturgeon (2), Turtle (3), Wabeno (2), Wakaw (1), Waskesiu (0-2).

EUNOTIACEAE

Eunotia gracilis (Ehr.) Rab. (15, p. 185, Fig. 253; 5, p. 217).

Deep (1), Greenwater (0-2), Sturgeon (3), Turtle (0-1).

Eunotia lunaris (Ehr.) Grunow (15, p. 183, Fig. 249; 5, p. 225).

Jackfish (0-1), Kingsmere (0-1), Murray (0-2).

ACHNANTHACEAE

Rhoicosphenia curvata (Kütz.) Grunow (15, p. 211, Fig. 311; 6, p. 240).

Atten (1), Bitter (1), Christopher (1), Echo (0-3), Fishing (0-1), Kenderdine (0-1), Last Mountain (0-2), Little Loon (1), Manito (0-2), Midnight (1), Murray (0-2), Sandy Beach (1), Wakaw (1), Witchehan (1).

Cocconeis pediculus Ehr. (15, p. 188, Fig. 259; 6, p. 244).

Echo (0-1), Pasqua (1), Turtle (0-1).

Cocconeis placentula Ehr. (15, p. 189, Fig. 260; 6, p. 244).

Birch (0-2), Clearwater (1), Deep (1), Echo (0-2), Greenwater (0-1), Helene (2), Kenderdine (0-2), Margo (0-1), Murray (0-2), Pasqua (1), Round II (1), Sandy Beach (2), Turtle (0-2), Wakaw (2).

NAVICULACEAE

Navicula amphibola Cleve (15, p. 309, Fig. 554; 6, p. 405).

Jackfish (0-1).

Navicula anglica Ralfs (15, p. 303, Figs. 530, 531; 6, p. 384).

Basin (2), Christopher (2), Echo (0-1), Fishing (0-2), Last Mountain (0-1), Little Loon (1), Little Quill (0-1), Murray (0-1), Round II (1), Soda (0-1), Turtle (0-1).

Navicula bacilliformis Grunow (15, p. 273, Fig. 446; 6, p. 369).

Echo (0-1).

Navicula cincta (Ehr.) Kütz. (15, p. 298, Fig. 510; 6, p. 384).

Broughton (1), Turtle (0-1).

Navicula cryptocephala Kütz. (15, p. 295, Fig. 496; 6, p. 383).

Atten (3), Big Quill (0-1), Brightsand (0-1), Broughton (1), Christopher (2), Deep (1), Emma (1), Fishing (0-2), Greenwater (0-1), Helene (2), Kenderdine (0-2), Last Mountain (0-2), Little Loon (1), Margo (0-1), Midnight (2), Murray (0-1), Turtle (0-2), Wakaw (2).

Navicula cuspidata Kütz. (15, p. 268, Fig. 433; 6, p. 366).

Bitter (1), Broughton (1), Echo (0-1), Emma (1), Helene (2), Kenderdine (0-3), Midnight (2), Murray (0-1).

Navicula cuspidata var. *ambigua* (Ehr.) Cleve (15, p. 268, Fig. 434; 6, p. 366).

Brightsand (0-2), Emma (1), Helene (2), Jackfish (0-1), Kenderdine (0-1), Little Loon (1), Murray (0-1), Stony (1).

Navicula dicephala (Ehr.) W. Smith (15, p. 302, Fig. 526; 6, p. 386).

Emma (1), Turtle (0-1).

Navicula exigua (Greg.) O. Muller (15, p. 305, Fig. 538).

Echo (0-2).

Navicula falaisiensis var. *lanceola* Grunow (15, p. 302, Fig. 525; 6, p. 400).

Atten (2), Margo (0-1).

Navicula gastrum Ehr. (15, p. 305, Fig. 537).

Brightsand (0-1), Emma (1), Fishing (0-1), Halkett (0-1), Helene (2), Pasqua (1), Waskesiu (0-1).

Navicula Grevillei (Ag.) Cleve (6, p. 376).

Little Quill (0-1).

Navicula hungarica var. *capitata* (Ehr.) Cleve (15, p. 298, Fig. 508; 6, p. 389).

Echo (0-2).

Navicula lanceolata (Ag.) Kütz. (15, p. 305, Fig. 540).

Brightsand (0-1), Helene (2), Little Loon (1), Turtle (0-2), Waskesiu (0-1).

Navicula oblonga Kütz. (15, p. 307, Fig. 550; 6, p. 395).

Bagwa (2), Birch (0-1), Brightsand (0-1), Broughton (1), Christopher (1), Echo (0-1), Emma (1), Fishing (0-1), Helene (2), Jackfish (0-2), Kenderdine (0-3), Margo (0-1), Murray (0-2), Soda (0-1), Turtle (0-2), Wakaw (1), Waskesiu (0-1), Witchehan (2).

Navicula pupula Kütz. (15, p. 281, Fig. 467a; 6, p. 369).

Kenderdine (0-3).

Navicula pusilla W. Smith (15, p. 311, Fig. 558; 6, p. 406).

Brightsand (0-1), Kenderdine (0-2).

Navicula pygmaea Kütz. (15, p. 312, Fig. 561; 6, p. 416).

Echo (0-2).

Navicula radiosa Kütz. (15, p. 299, Fig. 513; 6, p. 397).

Brightsand (0-1), Christopher (1), Crean (0-1), Deep (2), Emma (2), Greenwater (0-1), Helene (2), Last Mountain (0-1), Midnight (2), Murray (0-1), Round II (1), Stony (1), Sturgeon (2), Turtle (1-2), Wabeno (1), Wakaw (1), Waskesiu (0-1).

Navicula tuscule (Ehr.) Grunow (15, p. 308, Fig. 552; 6, p. 385).

Atten (1), Brightsand (0-1), Turtle (0-2).

Navicula viridula Kütz. (15, p. 297, Fig. 503; 6, p. 388).

Good Spirit (1), Kenosee (1).

Pinnularia major (Kütz.) Cleve (15, p. 331, Fig. 614; 6, p. 446).

Crean (0-1), Halkett (0-1), Margo (0-1), Waskesiu (0-1).

Pinnularia nobilis Ehr. (15, p. 337, Fig. 619; 6, p. 445).

Greenwater (0-1), Waskesiu (0-1).

Pinnularia viridis (Nitzsch) Ehr. (15, p. 334, Fig. 617a; 6, p. 446).

Bitter (1), Brightsand (0-1), Broughton (1), Clearwater (1), Crean (0-1), Greenwater (0-1), Halkett (0-1), Helene (1), Kenderdine (0-1), Last Mountain (0-1), Little Loon (1), Meeting (1), Midnight (1), Waskesiu (0-1).

Caloneis amphisbaena (Bory) Cleve (15, p. 230, Fig. 346; 6, p. 314).

Brightsand (0-1), Echo (0-1), Witchehan (1).

Caloneis amphisbaena var. **subsalina** (Donkin) Cleve (15, p. 230, Fig. 347; 6, p. 315).

Turtle (0-1).

Neidium iridis (Ehr.) Cleve (15, p. 245, Fig. 379; 6, p. 321).

Brightsand (0-1), Helene (2), Kingsmere (0-1), Sandy Beach (1), Soda (0-1), Sturgeon (1).

Neidium productum (W. Smith) Cleve (15, p. 245, Fig. 383; 6, p. 321).

Brightsand (0-1), Last Mountain (0-1), Little Loon (1), Margo (0-1).

Stauroneis phoenicenteron (Nitzsch) Ehr. (15, p. 255, Fig. 404; 6, p. 421).

Brightsand (0-1), Broughton (1), Crean (0-1), Helene (2), Jackfish (0-1), Little Loon (1), Margo (0-2), Murray (0-1), Soda (0-1).

Gyrosigma attenuatum (Kütz.) Cleve (15, p. 224, Fig. 330; 6, p. 455).

Crean (0-2), Halkett (0-2), Helene (1), Kingsmere (0-1), Meeting (1), Moose Creek (1), Turtle (0-1), Wabeno (1).

Gyrosigma Kuetzingii (Grunow) Cleve (15, p. 224, Fig. 333; 6, p. 461).

Meeting (1).

Pleurosigma elongatum W. Smith (15, p. 228, Fig. 343; 6, p. 470).

Bitter (1), Little Quill (0-2), Redberry (0-1).

Scoliopleura peisonis Grunow (15, p. 338, Fig. 622; 6, p. 361).

Atten (1).

Amphiprora alata Kütz. (15, p. 340, Fig. 625; 6, p. 483).

Bitter (1), Echo (0-1), Fishing (0-3), Last Mountain (0-1), Lenore (1), Margo (0-1), Redberry (0-1).

Amphiprora ornata Bailey (15, p. 340, Fig. 323; 6, p. 484).

Birch (0-1), Emma (1), Halkett (0-1), Midnight (1), Stony (1), Waskesiu (0-1).

Tropidoneis lepidoptera (Greg.) Cleve (6, p. 480).

Big Quill (0-1), Echo (0-1), Last Mountain (0-3), Little Quill (0-1), Pasqua (1), Stony (1), Stony (1).

Mastogloia Smithii Thwaites (15, p. 215, Fig. 314; 6, p. 332).

Bitter (2), Brightsand (0-1), Little Quill (0-1), Turtle (0-1).

Mastogloia Smithii var. **lacustris** Grunow (15, p. 217, Fig. 316; 6, p. 332).

Atten (3), Bitter (2), Sandy Beach (1).

GOMPHONEMATACEAE

Gomphonema capitatum Ehr. (6, p. 292).

Jackfish (0-2), Last Mountain (0-2), Madge (1), Murray (0-1), Redberry (0-1).

Gomphonema olivaceum (Lyngb.) Kütz. (15, p. 378, Fig. 719; 6, p. 296).

Christopher (1), Echo (0-2), Sturgeon (1), Turtle (0-2).

Gomphonema parvulum (Kütz.) Grunow (15, p. 373, Fig. 713a; 6, p. 294).

Echo (0-1).

CYMBELLACEAE

Cymbella affinis Kütz. (15, p. 362, Fig. 671; 6, p. 276).

Atten (1).

Cymbella aspera (Ehr.) Cleve (15, p. 365, Fig. 680; 6, p. 278).

Beartrap Creek (1), Brightsand (1), Deep (1), Kingsmere (0-1), Wakaw (1).

Cymbella cistula (Hempr.) Grunow (15, p. 363, Fig. 676a; 6, p. 280).

Brightsand (1-2), Crean (0-1), Deep (1), Echo (0-2), Emma (1), Jackfish (1), Meeting (1), Pasqua (1), Sturgeon (2), Turtle (1-2).

Cymbella cymbiformis (Kütz.) Van Heurck (15, p. 363, Fig. 672; 6, p. 279).

Atten (1), Brightsand (2), Christopher (2), Deep (1), Echo (0-1), Emma (1), Fishing (0-1), Halkett (0-1), Helene (2), Jackfish (0-2), Moose Creek (1), Murray (0-1), Pasqua (1), Redberry (0-1), Sturgeon (2), Turtle (2).

Cymbella Ehrenbergii Kütz. (15, p. 356, Fig. 656; 6, p. 275).

Last Mountain (0-1), Margo (0-1).

Cymbella lanceolata (Ehr.) Van Heurck (15, p. 364, Fig. 679; 6, p. 279).

Bitter (1), Brightsand (0-1), Echo (0-1), Helene (1), Jackfish (0-1), Meeting (1), Moose Creek (1), Murray (0-1), Sturgeon (2), Turtle (0-1), Waskesiu (0-1).

Cymbella naviculiformis Auers. (15, p. 356, Fig. 653; 6, p. 281).

Atten (1), Helene (1), Jackfish (0-1).

Cymbella parva (W. Smith) Cleve (15, p. 363, Fig. 675; 6, p. 281).

Brightsand (0-2), Turtle (0-2).

Cymbella prostrata (Berk.) Cleve (15, p. 357, Fig. 659; 6, p. 285).

Echo (0-1).

Cymbella pusilla Grunow (15, p. 354, Fig. 646).

Atten (3), Christopher (1), Turtle (1-2).

Cymbella tumida (Bréb.) Van Heurck (15, p. 366, Fig. 677; 6, p. 280).

Atten (1), Last Mountain (0-1), Little Manitou (0-2), Waskesiu (0-1).

Cymbella turgida (Greg.) Cleve (15, p. 358, Fig. 660; 6, p. 283).

Kenderdine (0-1), Last Mountain (0-1).

Amphora commutata Grunow (15, p. 345, Fig. 632).

Little Manitou (0-1), Little Quill (0-1).

Amphora ovalis Kütz. (15, p. 342, Fig. 628; 6, p. 254).

Atten (2), Birch (0-2), Brightsand (0-2), Christopher (1), Crean (0-1), Deep (1), Echo (0-2), Fishing (0-2), Greenwater (0-1), Helene (2), Jackfish (1-2), Kenderdine (0-2), Last Mountain (0-1), Little Loon (1), Little Quill (0-2), Margo (0-1), Meeting (1), Murray (0-2), Pasqua (2), Pelletier (1), Stony (1), Turtle (2), Wakaw (1), Witchekan (2).

Amphora proteus Greg. (6, p. 254).

Jackfish (0-1).

Epithemia argus Kütz. (15, p. 383, Fig. 727a; 6, p. 489).

Atten (1), Brightsand (2), Christopher (1), Kenderdine (0-2), Margo (0-1), Sandy Beach (1), Stony (1), Turtle (1-2).

Epithemia turgida (Ehr.) Kütz. (15, p. 387, Fig. 733; 6, p. 488).

Birch (0-1), Brightsand (2), Crean (0-1), Deep (1), Echo (0-2), Fishing (0-1), Greenwater (0-1), Halkett (0-1), Helene (2), Jackfish (0-1), Kingsmere (0-1), Little Loon (1), Margo (0-2), Murray (0-1), Pasqua (2), Round II (1), Turtle (1-2), Wakaw (1).

Epithemia zebra (Ehr.) Kütz. (15, p. 384, Fig. 729; 6, p. 490).

Birch (0-1), Bitter (1), Brightsand (0-2), Echo (0-2), Fishing (0-1), Helene (2), Pasqua (1), Wakaw (1), Witcheakan (1).

Rhopalodia gibba (Ehr.) O. Müller (15, p. 390, Fig. 740; 6, p. 491).

Beartrap Creek (3), Birch (0-2), Brightsand (2-3), Deep (1), Emma (2), Helene (3), Jackfish (0-1), Kenderdine (0-2), Kenosee (1), Last Mountain (0-1), Meeting (1), Murray (0-1), Stony (1), Turtle (2).

Rhopalodia gibba* var. *ventricosa (Ehr.) Grunow (15, p. 391, Fig. 741; 6, p. 491).

Beartrap Creek (2), Brightsand (2), Deep (1), Helene (3), Little Loon (1), Round II (1), Turtle (0-1).

NITZSCHIACEAE

Nitzschia acicularis W. Smith (15, p. 423, Fig. 821; 6, p. 525).

Last Mountain (0-2).

Nitzschia commutata Grunow (15, p. 405, Fig. 774).

Kenderdine (0-1).

Nitzschia hungarica Grunow (15, p. 401, Fig. 766; 6, p. 498).

Brightsand (0-1), Stony (1).

Nitzschia lanceolata W. Smith (6, p. 520).

Murray (0-1).

Nitzschia palea (Kütz.) W. Smith (15, p. 416, Fig. 801; 6, p. 521).

Brightsand (0-2), Christopher (2), Echo (0-3), Greenwater (0-2), Helene (2), Jackfish (0-1), Kenderdine (0-2), Meeting (1), Midnight (2), Murray (0-1), Stony (3), Turtle (2).

Nitzschia sigmoidea (Ehr.) W. Smith (15, p. 419, Fig. 810; 6, p. 513).

Brightsand (0-1), Christopher (1), Emma (1), Greenwater (0-1), Halkett (0-1), Helene (2), Jackfish (0-2), Last Mountain (0-1), Midnight (1), Murray (0-1), Redberry (0-1).

Nitzschia spectabilis (Ehr.) Ralfs (15, p. 419, Fig. 809; 6, p. 517).

Helene (1).

Nitzschia vermicularis (Kütz.) Grunow (15, p. 419, Fig. 811; 6, p. 514).

Christopher (1).

Hantzschia amphioxys (Ehr.) Grunow (15, p. 394, Fig. 747; 6, p. 528).

Beartrap Creek (1), Echo (0-1).

Denticula elegans Kütz. (15, p. 383, Fig. 725; 6, p. 530).

Atten (2), Brightsand (0-1), Echo (0-1).

SURIPELLACEAE

Cymatopleura elliptica (Bréb.) W. Smith (15, p. 426, Fig. 825; 6, p. 532).

Brightsand (0-1), Christopher (1), Echo (0-1), Emma (1), Helene (2), Jackfish (0-3), Little Loon (2), Margo (0-2), Meeting (1), Murray (0-1), Pasqua (1), Soda (0-1), Waskesiu (0-1).

Cymatopleura solea (Bréb.) W. Smith (15, p. 425, Fig. 823a; 6, p. 532).

Birch (1-2), Brightsand (1-2), Crean (0-1), Deep (1), Echo (0-1), Emma (1), Fishing (0-1), Halkett (0-1), Jackfish (1), Kenderdine (0-1), Kingsmere (0-1), Last Mountain (0-1), Little Loon (1), Little Manitou (0-1), Meeting (1), Moose Creek (1), Murray (0-1), Pasqua (1), Stony (1), Turtle (1-3), Waskesiu (0-2).

Suriella Baileyana MacKay (6, p. 539).

Big Quill (0-2), Bitter (2), Fishing (0-1), Jackfish (0-1), Last Mountain (0-1), Little Quill (0-3), Manito (0-1), Redberry (0-2).

Surirella biseriata Bréb. (15, p. 432, Figs. 831, 832; 6, p. 535).

Broughton (1), Halkett (0-1), Lavallee (1), Meeting (1), Waskesiu (0-1).

Surirella elegans Ehr. (15, p. 440, Figs. 858, 859; 6, p. 537).

Crean (0-1).

Surirella ovalis Bréb. (15, p. 441, Figs. 860, 861; 6, p. 541).

Big Quill (0-3), Crean (0-1), Fishing (0-2), Jackfish (0-2), Last Mountain (0-1), Little Manitou (0-1), Little Quill (2), Manito (0-1), Margo (0-1), Redberry (0-2), Sandy Beach (1), Stoney (1), Waskesiu (0-1).

Surirella ovata Kütz. (15, p. 442, Figs. 863, 864; 6, p. 541).

Atten (2), Basin (1), Big Quill (0-3), Birch (2-3), Deep (2), Fishing (0-1), Greenwater (1-2), Helene (2), Jackfish (0-3), Kenosee (1), Last Mountain (0-3), Lenore (1), Little Quill (2), Manito (0-3), Murray (0-1), Soda (0-1), Witchekan (1).

Surirella ovata var. **crumena** (Bréb.) Van Heurck (15, p. 443, Fig. 867; 6, p. 541).

Manito (0-3).

Surirella robusta Ehr. (15, p. 437, Fig. 850; 6, p. 537).

Jackfish (0-2), Waskesiu (0-1).

Surirella spiralis Kütz. (15, p. 445, Fig. 870).

Crean (0-1). A single but perfect specimen seen.

Campylodiscus clypeus Ehr. (15, p. 448, Fig. 873; 6, p. 552).

Atten (1), Bitter (1), Fishing (0-1), Jackfish (0-1), Kenderdine (0-1), Last Mountain (0-1), Manito (2-3), Soda (2), Sturgeon (1), Wakaw (1).

HETEROKONTAE

BOTRYOCOCCACEAE

Botryococcus Braunii Kütz. (30, p. 84, Pl. 15, Fig. 5; 24, p. 91, Figs. 71 to 75).

Big Quill (0-3), Birch (0-1), Christopher (2), Greenwater (0-1), Kenderdine (0-2), Last Mountain (0-2), Murray (0-1), Soda (1-3), Stony (1), Sturgeon (1), Wabeno (1), Witchekan (3).

CHLOROTHECIACEAE

Characiopsis sp.

Big Quill (0-2), Bitter (1), Broughton (1), Kenderdine (0-1), Last Mountain (0-1), Little Quill (0-1), Manito (0-1), Murray (0-2), Redberry (0-2), Soda (0-2), Stony (1), Sturgeon (1), Waskesiu (0-1).

The specimens agree in cell outline with *C. saccata* Carter as described and figured by Pascher (24); it agrees especially with Fig. 53M, except for a longer stipe and less acute apex. The cells are 30 to 60 μ in width and 320 to 560 μ in length; length of stipe 25 to 40 μ . Unfortunately Pascher fails to give any dimensions, and the war in Europe makes it impossible to consult the original description. The Saskatchewan specimens may be new to science; but until more perfect material, especially in the immature stages, is available, the form is best left unnamed.

TRIBONEMATACEAE

Tribonema minus (Wille) Hazen (30, p. 87, Pl. 15, Figs. 17, 18).

Last Mountain (0-3), Little Quill (0-2), Manito (0-1).

CHRYSTOPHYCEAE

OCHROMONADACEAE

Dinobryon bavaricum Imhof (30, p. 73, Pl. 13, Fig. 10).

Christopher (1), Kingsmere (0-3), Shady (1), Waskesiu (0-1).

Dinobryon divergens Imhof (30, p. 75, Pl. 14, Fig. 2; 26, p. 8, Pl. 1, Fig. 4).

Atten (1), Birch (0-2), Bitter (2), Brightsand (1-2), Christopher (1), Crean (1-3), Deep (3), Edwards (1), Emma (2), Fishing (0-2), Greenwater (0-3), Halkett (1-2), Helene (2), Jackfish (0-3), Kenderdine (0-1), Kingsmere (0-4), Little Loon (4), Meeting (2), Moose Creek (1), Murray (2-4), Pelletier (3), Round I (1), Sturgeon (2), Tibiska (2), Turtle (3), Wabeno (3), Waskesiu (0-3).

Dinobryon sertularia Ehr. (30, p. 74, Pl. 13, Fig. 17, Pl. 14, Fig. 1; 26, p. 8, Pl. 1, Fig. 6).
Crean (0-2), Kingsmere (0-2).

Dinobryon stipitatum Stein (30, p. 74, Pl. 13, Fig. 11; 26, p. 8, Pl. 1, Fig. 7).
Brightsand (1-3), Emma (3).

DINOPHYCEAE

DINOFLAGELLATAE

Glenodinium pulvisculus (Ehr.) Stein (9, p. 286, Fig. 21).
Fishing (0-2), Halkett (0-2), Midnight (1).

Peridinium Cunninghami (Lemm.) Lemm. (9, p. 292, Figs. 32a to d).
Clearwater (1).

Peridinium inconspicuum Lemm. (9, p. 294, Figs. 37a to d).
Basin (2).

Peridinium tabulatum (Ehr.) Clap. and Lachm. (9, p. 298, Figs. 46a to e; 26, p. 35, Pl. 10, Figs. 1, 2).

Atten (1), Birch (0-1), Christopher (1), Crean (0-2), Edwards (1), Emma (1), Fishing (0-1), Halkett, (0-2), Kenderdine (0-2), Kingsmere (0-1), Lavallee (3), Lenore (1), Murray (0-1), Pelletier (1), Round I (2), Round II (1), Stoney (1), Stony (1), Tibiska (2), Wabeno (1), Waskesiu (0-1).

Ceratium hirundinella (O.F.M.) Schrank (9, p. 303, Figs. 58 a to f; 26, p. 33, Pl. 8, Fig. 2).

Atten (2), Basin (3), Beartrap Creek (1), Big Quill (0-2), Birch (0-1), Brightsand (2), Broughton (2), Carlyle (1), Christopher (2), Clearwater (1), Crean (0-3), Deep (2), Echo (1-3), Edwards (1), Emma (3), Fishing (1-3), Good Spirit (3), Greenwater (1-3), Halkett (2-3), Helene (2), Jackfish (0-3), Kenderdine (1-3), Kenossee (2), Kingsmere (0-4), Last Mountain (0-3), Lavallee (3), Little Loon (3), Little Manitou (0-1), Little Quill (0-2), Madge (2), Manito (1), Margo (0-2), Meeting (2), Midnight (1), Moose Creek (1), Murray (2-3), Pelletier (1), Redberry (1-2), Round I (4), Round II (2), Sandy Beach (1), Soda (1-2), Stony (2), Sturgeon (2), Tibiska (3), Turtle (2-3), Wabeno (2), Waskesiu (0-4).

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STUDIES ON THE CHROMOSOME SPIRALIZATION CYCLE IN *TRILLIUM*¹

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Abstract

Studies of the major, relic, and relational coils and of chromosome, chromatid, and chromonema length changes in meiotic, microspore, and root tip chromosomes of *Trillium erectum* L. and *T. grandiflorum* Salisb. have led to the following conclusions:—

Elongation of the chromonema occurs between early diakinesis and first anaphase and between second anaphase and microspore prophase. Contraction occurs between zygotene and early diakinesis, between first and second anaphase, and during microspore prophase. Chromonema elongation between early diakinesis and anaphase is associated with the formation of the major coil and its transition into the relic coil of the microspore prophase is accompanied by a further elongation. The tertiary split results in the half-chromatids being associated in the form of a plectonemic spiral, which persists as such to microspore prophase. The gyres of the plectonemic relic coil become partially straightened out to form a relational coil, whose twists are in the same direction as their antecedent relic coils. During microspore prophase some relational twists are apparently eliminated at intrabrachial changes of direction, others by contraction and untwisting of the chromatids.

Changes of direction fall into three categories: (1) those associated with attachments, (2) those associated with chiasmata, and (3) the remainder, the frequency of which is proportional to the number of gyres. Intrabrachial changes of direction are more numerous in microspore prophase than in root tip chromosomes, presumably because Factor (2) is inoperative in the latter. There are very few data that could possibly be taken to indicate that chromosomes may have an inherent directional pattern of coiling.

To the tentative hypothesis of Wilson and Huskins that the major coil of meiosis is formed by an elongation of the chromonema within a restricted space, the pellicle, the following may be added: the half-chromatids at metaphase are wound in the form of a plectonemic spiral. The straightening out of this spiral results in the relationally twisted chromatids of microspore prophase. These twists are eliminated both by cancellation at the points at which changes of direction have occurred in the major coil and also by contraction and untwisting of the chromatids.

Introduction

The spiral structure of chromosomes has been the subject of much study and speculation in recent years. Darlington (5, 8), Kaufmann (23), Huskins (17), Geitler (13), Straub (46), Nebel (35, 36), Wilson and Huskins (50), Kuwada (25), and Kuwada and Nakamura (28) have together presented or discussed most of the current hypotheses regarding the nature and mechanics of chromosome coiling.

Nebel (35) has grouped the proponents of the various theories on the mechanics of coiling into two schools which he terms "matrical" and "molecular". This division recognizes the emphasis that one group lays on the

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role of the immediate environment or "matrix" of the chromonema in causing coiling and the emphasis that the other lays on the idea of a molecular reorientation within the chromonema. Most workers, however, have realized that internal and external factors must be to some extent interactive; they have differed in the relative emphasis placed on these.

The spiralization theory of Darlington (5, 8 (p. 483 for latest formulation)) postulates "the formation of a molecular spiral, a spiral torsion within the thread," which produces the visible spirals. A detailed mechanism similar in principle has been suggested by Nebel (35). Huskins and Smith (19) suggested that heterogonic growth of a coiled chromonema might be a factor in the production of the succeeding coil and Kuwada (24) postulated anisotropic swelling of the chromonema resulting in a waviness that becomes a spiral owing to contraction of the surrounding matrix. Sax (39) and Sax and Humphrey (41) from observations on *Secale* and *Tradescantia* express the opinion that "coiling of the chromonema is due to a differential rate of contraction between the chromosome and the chromonema" (41). Wilson and Huskins (50) showed that in *Trillium* the chromonema elongates while the length of the chromosome remains almost unchanged.

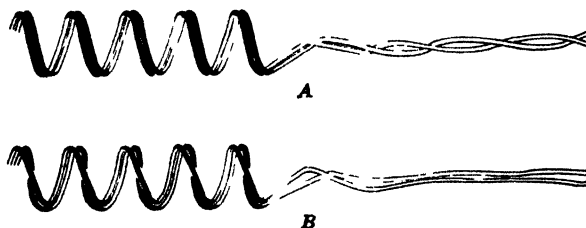
The large-gyred spiral of the meiotic chromonemata is now generally termed the "major spiral" (19). Each of the four chromatids of a bivalent chromosome constitutes such a spiral, but in some materials, for example *Tradescantia*, each pair of sister chromatids may be coiled in common and so closely associated that they are often difficult to resolve. In some pollen mother cells of asynaptic *Trillium erectum* each chromatid forms a completely independent spiral, whereas in normal *T. erectum* the chromatids are semi-independent (50).

A small-gyred spiral with its axis perpendicular to that of the major spiral is described by most workers. It constitutes an integral part of the spiralization mechanism of Darlington (5). Huskins (17) casts doubt on the existence of this structure as a regular spiral and sees clear evidence only of waviness along the chromonema. This interpretation is compatible with the "minor spiral" of Nebel and Ruttle (37) but not, in our opinion, with that of the Fujii-Kuwada school or that of Darlington which is based on a diagram, Fig. 3, of Kuwada and Nakamura (26). Actually, as the authors have since recognized (personal communication), this diagram did not represent accurately the observations as described in the text.

Somatic chromosomes have now clearly been shown to have a coiled structure like that of the major spiral of meiosis but they are generally of smaller diameter (12, 13, 14, 2). This somatic spiral has sometimes been termed a "minor spiral" and has been identified by some authors with the minor spiral of meiosis (40, 8). Some confusion has in consequence resulted, especially as different hypotheses of spiralization relate the three kinds of spirals in different ways. Until their ontogenetic relationship is established, clarity is best preserved by referring to the spiral in the somatic chromosome as a "standard coil," Nebel (36), or simply as a somatic coil.

The two chromatids of a prophase somatic chromosome are twisted about each other (i.e., they are extended interlocked coils turning in the same direction) forming what Darlington (5) has termed a "relational coil." His hypothesis, which includes a mechanism of crossing-over, postulates that the paired chromosomes of pachytene are also relationally coiled. The observational evidence for this is less secure.

In *Trillium erectum* and some other plants without prolonged meiotic interkinesis, the major spirals of the second division are continuous in structure with those of the first division. Similarly the large-gyred spirals of microspore prophase are obviously derived from the major spirals of meiosis and Darlington (5) has aptly termed them "relic spirals". Darlington's "super-spirals", the larger waves that may be superimposed upon the relic spiral, are less definite structures.



TEXT-FIG. 1. (A) A plectonemic coil ("double-stranded" orthospiral). (B) A paranemic coil ("double-stranded" anorthospiral). In the former the component halves are interlocked and form a relational coil when the gyres are partially straightened out. The two spirals of a paranemic pair are free to separate and therefore give independent strands when straightened out.

Kuwada (25) describes two types of double-stranded spirals: (1) orthospirals, which are formed when the two threads being coiled have one end free so that internal twisting does not occur; (2) anorthospirals, which result when two strands with both ends fixed are coiled together and in consequence have a twist compensating for each gyre of the spiral (Text-fig. 1). Orthospirals are interlocked and cannot be separated without untwisting; anorthospirals are independent and can readily be pulled apart or fitted into each other. When a pair of orthospirals is straightened out, it will give a pair of relationally twisted strands, whereas anorthospirals will give two independent strands. The term "paranemic" (*para*=beside) instead of anorthospiral will be used here since it is simpler and its implications are clear. Instead of orthospiral "plectonemic" (*plektos*=twisted) will be used as this has the advantage of indicating the relationship of the strands both when in the major coil and when straightened out into the relational coil, twisted in the same direction, to which, as will be shown, it gives rise. Observations of relational coiling have been made by Darlington (5, 7), Sax (40), Husted (21, 22), Upcott (47), and Csik and Koller (3) but none of them has presented any very definite evidence or hypothesis of its mode of origin.

Data on the molecular orientation within coiled chromosomes have been sought in studies with polarized light (27, 34, 42, 43, 44), but the evidence

in so far as it directly concerns coiling is too equivocal to be in any sense satisfactory. Apart from these studies, hypotheses relating coiling to molecular structure have relied on analogy or deductive chains of reasoning. Interpretation of the minor spirals which are near the limit of visibility has naturally varied considerably with the optical equipment, wave length of light, methods of fixation, and the material used, as well as with the opinions of the observers on other related problems such as the number of chromonemata within a chromosome or the time of "splitting". The authors have felt that before speculation proceeds further from such indefinite data, observations capable of statistical treatment should be made on larger structures about which little or no difference of opinion can exist in matters of interpretation. From such observations more rigid limiting requirements for hypotheses dealing with the smaller or submicroscopic structures can be laid down. So far such studies have comprised analyses of changes of direction in the large-gyred, "major" spiral of meiosis (20), of chromosome and chromonema length (50), and of the relationship of chromatids at successive chiasmata (18). These all follow on the general analysis of meiosis and preliminary work on changes of direction in *Trillium erectum* initiated by Huskins and Smith (19). The present study embodies (a) an extension of the work on length changes, (b) a detailed analysis of the coiled structure found in three successive divisions (two of meiosis and the first of the microspore), with special regard to the relationship between certain characteristics of the meiotic major coil and the origin of the microspore "relational coil", and (c) a comparison of microspore relational coils with those in mitotic chromosomes of the root tip. From these studies it has definitely been possible to rule out certain concepts which have, through constant repetition, gradually been acquiring acceptance as observational data. Further, it has already been possible to build up inductive hypotheses covering parts of the problem. Deductive hypotheses remain necessary at present for other parts, but we are becoming encouraged to think that an inductive¹ approach to the whole problem of the mechanism of mitosis and of the relation of somatic mitosis to meiosis is more possible than has sometimes been assumed.

Materials and Methods

Rhizomes of *T. erectum* are collected near Ste. Agathe, Que., in September or October of each year, at which time their pollen mother cells are in pre-meiotic stages—they normally undergo meiosis in the late winter or very early spring. *T. grandiflorum* Salisb. was collected on Ile Perrot, Que., in early August. It ordinarily undergoes meiosis during September. The rhizomes were kept under various temperatures and conditions (Table I) until used. The time of onset of meiosis can be controlled to a considerable degree by temperature, but cannot be delayed indefinitely without actual freezing—some have undergone meiosis at temperatures as low as 2° C.

¹ It is realized, of course, that there is no sharp line of demarcation between deductive and inductive methods but these terms are used to distinguish the two schools that exist in cytogenetics as in physics, cf. Dingle et al. (11).

Material was prepared according to 2BD-crystal violet, aceto-carmine, and Feulgen techniques. The 2BD-crystal violet schedule, essentially that employed by Huskins and Smith (19), gave excellent spiral structure in *T. erectum* but comparatively poor results in pollen mother cells of *T. grandiflorum*. The best preparations of various stages during the first division of the microspore were obtained by smearing fresh anthers, then fixing with 3 : 1 alcohol-acetic fluid in a partial vacuum for three to seven minutes, replacing the fixing fluid with 45% acetic acid and staining with iron aceto-carmine (45). Warmke's (48) alcohol-hydrochloric acid method was used successfully to remove microspore walls where this was deemed necessary. Root tips were fixed in 3 : 1 alcohol-acetic at 60° C. and stained by the Feulgen method.

Observations were made with Zeiss 1.5 mm., 1.3 N. A. and 3 mm., 1.4 N. A. objectives combined with 7×, 15× and 20× oculars. All drawings used for measurements were made with the camera ludica at magnifications of 4000× and 3700×. All lengths given are totals for a complement of five chromosomes unless otherwise stated. For the most part photomicrographs were taken with a 7× ocular and a 3 mm., 1.4 N. A. objective.

The following formula has been used in calculating lengths of coiled chromonemata:

$$L = n\sqrt{p^2 + (\pi d)^2}$$

where L = the length of the chromonema, n = the number of gyres, p = pitch, and d = the gyre diameter measured as the outside diameter of the coil minus the diameter of the chromonema. Both diameters were the means of not less than five measurements per cell. In an earlier study (50) the magnification of errors that may arise through the use of this formula was pointed out and one-plane measurements were favoured. In the present study, particularly careful checking of the diameter of the major coil and of the chromonema itself has been carried out to minimize this effect. Although lengths (except means) have been given to the nearest micron, it should be emphasized that it is not considered that the measurements may not have an experimental error within such small limits. The authors agree with Manton (30) that exact determination of chromosome length is extremely difficult and consequently regard the data not as precise measurements but rather as close approximations. When they are used as relative lengths it is possible to reach conclusions on certain problems; the present paper is concerned with these rather than with the exact determination of length per se.

As measurements were made from both aceto-carmine and 2BD-crystal violet preparations, it was deemed necessary to determine the relative degree to which these two fixatives alter the dimensions of the chromosome and chromonema. It is readily apparent that, relative to 2BD, acetic acid fixation swells both chromosomes and chromonemata. An estimate of the resultant difference in chromonema length has been obtained by comparing first anaphase lengths from 2BD-crystal violet and aceto-carmine preparations made from the same bud at the same time. These give a conversion factor

of 1.6. Since this factor is only an approximation and may be inaccurate when extended to other stages, it should be pointed out that the main conclusions are not dependent upon the validity of the above factor, as there are enough data on material from the same fixative from which to draw most of the conclusions.

Observations

Length Changes

It has been shown by Wilson and Huskins (50) that the chromonema elongates during the formation of the major coil of meiosis. Further data have now been obtained on various stages in the development of the major coil, and the studies have been extended through the second division and the prophase of the first division of the microspore to include later length changes, unravelling of the relic coil, and elimination of relational twisting.

A résumé of chromosome and chromonema lengths at various stages of meiosis and first microspore division is given in Table I. Except for an apparent elongation between leptotene and zygotene, contraction occurs during meiotic prophase, reaching a maximum at early diakinesis. Chromonema elongation occurs between early diakinesis and first anaphase followed by a slight contraction before second anaphase. By early microspore prophase further elongation has occurred. This is followed by the usual prophase contraction presumably associated with elimination of relic and relational coiling and the formation of a new somatic coil.

Following this brief outline of length changes a detailed consideration of the various stages will be given. Lengths at early meiotic stages were calculated from the illustrations of Huskins and Smith (19). Pachytene lengths were measured from their drawings of whole chromosomes whereas zygotene and leptotene lengths were estimated from the interchromomeric distances in sections of chromosomes relative to these distances at pachytene. These estimates show an increase from 920μ at leptotene to 1040μ at zygotene followed by a decrease to 640μ at pachytene. This contraction ratio of 16 : 10 agrees remarkably well with the 15 : 10 ratio for corresponding stages found by Manton (30) in *Osmunda*. By diakinesis the chromosome is reduced to one-tenth of its zygotene length. This contraction, unlike that of somatic prophases is not accompanied by any visible coiling within the chromosome.

The relationship of length changes between diakinesis and anaphase and the development of the major spiral have been discussed by Wilson and Huskins (50). Further observations on 140 diakinesis and 80 first anaphase chromosomes from four different plants of *T. erectum* (Figs. 1, 2, 3) are presented in Table II. In each case, the mean chromonema length increases from diakinesis to anaphase whereas the mean chromosome length decreases slightly. When all the data are considered together the mean difference between chromonema lengths at the two stages is about 20 times the standard error.

Coefficients of variability for diakinesis and first anaphase chromonema lengths have been calculated from all complements measured. The vari-

TABLE I

CHROMOSOME AND CHROMONEMA LENGTHS* AND DETAILS OF MATERIAL

Stage	Chromatid or chromosome		Chromo- nema		Material and slide number	Year collected	Approximate temperature during:	
	A.C.	2BD	A.C.	2BD			Meiosis	Microspore mitosis

Trillium erectum

In pollen mother cells:								
Leptotene	—	—	920	575	H. & S. (19) Pl. I, Fig. 1			
Zygotene			1040	650	H. & S. (19) Pl. I, Fig. 2			
Pachytene			640	400	H. & S. (19) Text-fig. 3a, c, f			
Diplotene, late			186	116	H. & S. (19) Text-fig. 2a			
Diakinesis, mid	104	65	160	100	H. & S. (19) Pl. I, Fig. 8 (corrected for chromosome A)			
early	86	54	109	68	58-4E-1 chrs. slightly coiled	1937	12° C.	
early	106	66	133	83	58-5d-3 chrs. slightly coiled	1937	16° C.	
mid	126	79	175	109	65-L2-a chrs. half coiled	1938	20-22° C.	
mid	125	78	187	117	66-T-81c chrs. half coiled	1939	8-10° C.	
Metaphase I	99	62	320	200	H. & S. (19) Pl. III, Figs. 22, 23			
Anaphase I	99	62	320	200	H. & S. (19) Pl. II, Fig. 18			
	86	54	284	177	58-4E-1	1937	12° C.	
	91	57	318	199	58-5d-3	1937	16° C.	
	112	70	320	200	65-L2-a	1938	20-22° C.	
	90	68	313	197	66-T-81-b and -c	1939	8-10° C.	
	86	54	368	230	66-T-72	1939	3-4° C.	3-4° C.
	93	58	342	214	66-T-69-A	1939	3-4° C.	
	84	52	349	218	66-T-89-A	1939	3-4° C.	
Anaphase II	85	53	292	183	58-5d-3	1937	16° C.	
	72	45	303	189	66-T-72	1939	3-4° C.	3-4° C.
	72	45	334	209	66-T-87-1, B	1939	3-4° C.	
In microspores:								
Early prophase	549	343	1000	625	66-T-117-A	1939	4-7° C.	13-14° C.
	452	282	—	—	66-T-72	1939	3-4° C.	3-4° C.
Metaphase	110	69	—	—	63-RC	1938	3° C.	18-22° C.
	79	49	—	—	66-T-72-SB	1939	3-4° C.	3-4° C.
Additional slides used in analyses of changes of direction					58-2E-1	1937	4° C.	
					58-5H-3	1937	16° C.	

Trillium grandiflorum

In pollen mother cells:								
Anaphase I	98	61	375	234	69-G-34	1939	3-4° C.	
			394	246	69-G-110	1939	?	20-23° C.
In microspores:								
Early prophase	467	292			69-G-100 and -104	1939	?	20-23° C.
Metaphase	80	50			69-G-93, -94, -100, -104	1939	?	20-23° C.

NOTE: Figures in bold-face type were calculated by use of the 2BD-aceto-carmin factor 1.6

*All lengths are given in microns and are totals for a set of five.

TABLE II
CHROMOSOME AND CHROMONEMA LENGTHS AT DIAKINESIS AND FIRST ANAPHASE
(From material fixed in 2BD)

Material	Mean chromosome length	Mean chromonema length	Number whole sets	Total number of chromosomes					Mean number gyres	Mean diameter	Mean chromosome length	Mean chromonema length	Number whole sets*	Ratio diakinesis: anaphase lengths	
														Chromosomes	Chromonemata
				A	B	C	D	E							
Early diakinesis															
58-4E-1	54.3	67.6	5	6	6	6	5	6	43.2	1.25	53.5	177.0	4	0.98	2.62
58-5d-3	66.4	83.2	13	14	15	13	14	14	45.8	1.35	57.3	199.2	5	0.87	2.40
Mid-diakinesis															
65-L-2a	78.8	109.0	5	5	5	5	5	5	59.2	1.0	69.5	200.0	2	0.88	1.83
66-T-81c	77.6	116.6	5	5	5	5	5	5	60.2	1.0	68.2	196.8	5	0.88	1.70

Mean chromonema length in 28 complete complements at diakinesis = $90.0\mu \pm 3.6$; $v = 21.0 \pm 2.9$.

Mean chromonema length in 16 complete complements at anaphase = $194.0\mu \pm 4.4$; $v = 8.7 \pm 1.6$.

Mean chromonema length in 28 complete complements at diakinesis = $90.0\mu \pm 3.6$; $v = 21.0 \pm 2.9$.
Mean chromonema length in 16 complete complements at anaphase = $194.0\mu \pm 4.4$; $v = 8.7 \pm 1.6$.

*No odd chromosomes were analysed at anaphase as in diakinesis.

ability at diakinesis is significantly greater (four times the standard error) than at anaphase, despite the fact that no late diakinetik stages were measured. This indicates clearly that the chromonema undergoes a greater length change during diakinesis than at anaphase. Chromonema length is more difficult to determine accurately in first metaphase chromosomes. The data so far accumulated indicate that it is approximately the same as at anaphase. It is therefore reasonable to assume the correctness of the general impression gathered earlier, that it is chiefly during diakinesis that elongation between diplotene and anaphase takes place.

A determination of the exact amount of elongation between diakinesis and anaphase is hampered by two factors. The first is the fact that a waviness, obvious in the chromonemata of anaphase chromosomes, has not been taken into account in measuring lengths. The second difficulty is that, apart from errors of measurement, ratios determined from comparisons of any but the earliest and latest stages of elongation will obviously always be underestimates of the total elongation. Anaphase length being relatively stable, variations in the anaphase/diakinesis ratio will be upward to an extent dependent upon the earliness of the stage of diakinesis measured. The ratio varies from 1.70 to 2.62 (Table II), when determined from the mean diakinesis and anaphase lengths in each of the four plants examined. It ranged as high as 3.2 in measurements in individual pairs of diakinesis and anaphase complements from single plants. It seems reasonable to conclude, therefore, that, ignoring the minor waviness, chromonema elongation between earliest diakinesis and first anaphase is not less than threefold.

Mean chromosome lengths are shorter at anaphase than diakinesis in all four materials of Table II. The ratios of anaphase to diakinesis lengths are not, however, significantly less than one, and chromosome contraction is, therefore, not statistically established. If there is a real change it is obscured by the high variability in chromosome length in different cells (correlated in part with diameter). Further, at diakinesis, opening-out between chiasmata reduces chromosome length if measured as the shortest distance from end to end, and efforts to take this factor into account by measuring loops along the chromatids failed to give significant results since they introduced further variability. The present data, although failing to show a significant change in length do not definitely rule out the possibility of a chromosome contraction between diakinesis and anaphase but indicate that if such a contraction does occur it must be of very limited magnitude.

As there is normally no interkinetic resting stage in *T. erectum* or in *T. grandiflorum* the major coil of first anaphase persists, through second anaphase (Fig. 4) with no marked change in the structure of the coil. However, when both stages are measured in cells from the same plant (Table III, 66-T-72), gyre number, chromonema length, and mean gyre length are all significantly less at second anaphase than at first, showing that a slight contraction has occurred. Chromonema lengths were also measured at *either* first or second anaphase in three other rhizomes (Table III)

which had been kept under the same conditions as 66-T-72. The data as a whole show the chromonemata to be shorter at second than at first anaphase but when different plants are compared the difference is not always statistically significant.

TABLE III

LENGTHS OF CHROMONEMATA AT ANAPHASE AND OF CHROMATIDS AT MICROSPORE PROPHASE

Species	Plant No	Number of complete cells	Stage	Mean number of gyres	Mean chromonema or chromatid length	Mean gyre length
<i>T. erectum</i>	66-T-72	5	Anaphase I	63.6 ± 0.6	368 ± 4.5	5.79 ± 0.05
	66-T-69	5	Anaphase I	64.8 ± 1	342 ± 11	5.29 ± 0.21
	66-T-89	5	Anaphase I	63.6 ± 0.9	349 ± 6	5.49 ± 0.16
	66-T-72	10	Anaphase II	61.9 ± 0.6	304 ± 4.3	4.88 ± 0.10
	66-T-87	10	Anaphase II	59.4 ± 0.3	334 ± 4.7	5.62 ± 0.09
	66-T-72	10	Microspore prophase	57.2 ± 0.7	452 ± 12	7.92 ± 0.16
	61-T-117	7	Microspore prophase	60.6 ± 1.2	549 ± 25	9.08 ± 0.24
<i>T. grandiflorum</i>	69-G-110	5	Anaphase I	62.6 ± 1.6	394 ± 12	6.32 ± 0.21
	69-G-34	5	Anaphase I	65.4 ± 1.1	234 ± 7*	3.59 ± 0.11*
	69-G-100,-104	10	Early microspore prophase	52.2 ± 2.1	375 ± 11**	5.74 ± 0.18**
	69-G-104	6	Later prophase	47.5 ± 1.5	370 ± 8	7.93 ± 0.18

*Lengths for 69-G-34 are from 2BD-crystal violet preparations. Multiply by 1.6 to convert to aceto-carmin equivalents.

**Calculated by use of the 2BD-aceto-carmin factor 1.6.

The matrix¹ that envelops the major coil disappears after the second division of meiosis and a new matrix that follows the turns of the old major coil develops around each of the former half-chromatids. The relic coils of microspore prophase (Figs. 10, 11, 12, 19) persist to some degree until late prophase or early metaphase (Figs. 14 to 17), and the "chromatid lengths" are measured along the gyres of one of the two relationally coiled chromatids of each chromosome. Within these prophase chromatids wavy or loosely coiled chromonemata can be seen in some parts of the chromosomes of some preparations (Figs. 10, 11, 13, 26, 27). It has not been possible to differentiate chromonemata and matrix sufficiently clearly to obtain accurate measurements of chromonema length. From measurements of short segments they are estimated to be approximately the same length as zygotene chromonemata, i.e., about 1000μ, whereas the maximum chromatid length was 652μ, mean = 549μ (Table III, 66-T-117, the lower mean for 66-T-72 is presumably due to the fact that the measured cells were fixed at a later stage).

¹ The authors do not take seriously Darlington's (6) diatribe against the use of the term matrix, though they agree with him that it has often been used too loosely. Modern studies of ultra-violet absorption spectra may lead to a clarification of the nature and formation of the "matrix". Meanwhile nothing is gained by ignoring its existence or by devising new terms such as kalymma (15), or perinema (38).

In addition to chromatid length the mean number of gyres and mean gyre lengths are given in Table III for early prophase nuclei for two rhizomes of *T. erectum*. Chromatid and mean gyre lengths in early prophase of the first microspore division are significantly greater than the corresponding measurements at either meiotic anaphase, even in comparisons between different plants. Differences in the mean number of gyres at meiosis and microspore prophase are not always significant in comparisons of different plants, but in the single case in which gyre number was obtained at meiosis and also at microspore prophase (66-T-72), there is a small but significant decrease. It is obvious that if elongation occurs at the time that gyres are being lost the mean gyre length must increase. This could be effected by an increase either in pitch or in gyre diameter, or by a combination of both. Apparently both occur in the transition of the major coil to the relic during the interval between second anaphase and microspore early prophase of *T. erectum*.

Similarly, chromonema and chromatid lengths have been measured in *T. grandiflorum* (Table III). The difference between anaphase chromonema and microspore prophase chromatid lengths is again significant, although anaphase lengths which vary relatively little are being compared with those in prophase stages which are undergoing contraction and hence are highly variable. At a relatively early stage of microspore prophase the chromatid has again contracted to less than the length of the chromonema at meiotic anaphase. The full extent of the elongation after second anaphase will therefore be indicated only when the earliest prophase stages are measured and again almost all attempts to measure it will give underestimates.

Relational Coiling

The relic coils of early prophase usually show their two chromatids optically separate for short distances only and these mostly at the ends (Fig. 10, lower left). However, as prophase advances the doubleness becomes increasingly clear throughout (Figs. 13, 15, 20, 21) and it is seen that the two chromatids of the relic coil form a plectonemic spiral (Text-fig. 1A). During early prophase contraction the gyres decrease in both size and number and by mid- to late prophase relatively few remain (Figs. 17, 22, 23). Although some may be lost by unwinding it is obvious that for the most part gyres are being straightened out faster than their chromatids are untwisting. This gives rise to the relational twisting typical of late prophase (Figs. 15, 16, 22, 23, 28). The relational coil thus formed is, of course, in the same direction as the relic from which it was derived. The earliest stage at which direction of the relational twists could be determined in all chromosomes was about mid-prophase or, more precisely, at a chromatid length of about 200-250 μ .

Fifty-four complete microspore complements of *T. grandiflorum*, at stages varying from mid-prophase to metaphase, have been analysed for chromatid length, number and direction of relational twists, and mean intertwist distance. They were arranged in order of descending length and divided, for brevity in presentation, into four equal groups, totals and means for which are given

in Table IV. Results for the *E* chromosomes are given separately so that comparisons can be made with root tip data in which only this, the longest chromosome, was studied. The steady reduction in number of twists as length decreases is obvious (Text-fig. 2). If length is taken as an index of the stage of mitosis, then the amount of relational coiling is found to be highly variable at all stages measured. Individual microspores ranged in total chromatid length from 214μ at mid-prophase to 63μ at metaphase, and the number of twists from 48 to 0. Between the first and fourth group the mean length drops from 179.6 ± 4.9 to 84.1 ± 4.3 and the mean number of twists from 30.0 ± 2.7 to 5.0 ± 1.4 .

TABLE IV

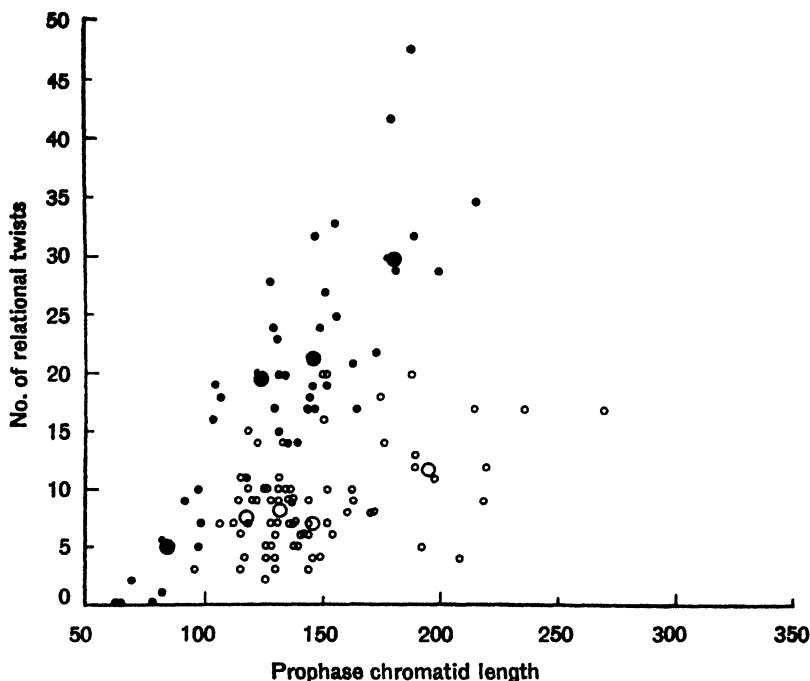
CHROMATID LENGTHS,* NUMBER OF TWISTS, NUMBER OF INTRABRACHIAL CHANGES IN DIRECTION, AND MEAN INTERTWIST DISTANCE IN THE RELATIONAL COIL OF MICROSPORE AND ROOT TIP CHROMOSOMES OF *T. grandiflorum* AND *T. erectum*

Material	Number of cells (n)	Total length (L)	Number of twists (T)	Number of changes	L/n	T/n	L/T + A**
<i>T. grandiflorum</i>							
Microspores							
Group 1	11	1976	330	45	179.6 ± 4.9	30.0 ± 2.7	5.1
Group 2	11	1601	234	30	145.5 ± 1.6	21.3 ± 2.1	5.5
Group 3	11	1359	215	32	123.2 ± 3.1	19.6 ± 1.4	5.0
Group 4	11	926	55	1	84.1 ± 4.3	5.0 ± 1.4	8.4
Total	44	5862	834	108	133.2 ± 5.6	18.9 ± 1.7	6.6
Chr. E	44	1668	266	38	37.9 ± 1.6	6.0 ± 0.6	5.4
Root tip							
Chr. E	50	1844	252	15	36.9 ± 0.5	5.0 ± 1.0	6.1
<i>T. erectum</i>							
Microspores*							
Group 1	18	3494.4	212	36	194.1 ± 6.8	11.8 ± 1.1	11.5
Group 2	18	2612.8	128	9	145.2 ± 1.3	7.1 ± 0.7	11.9
Group 3	18	2371.2	146	9	131.7 ± 0.8	8.1 ± 0.7	10.0
Group 4	18	2112.0	135	10	117.3 ± 1.8	7.5 ± 0.9	9.4
Total	72	10,590.4	626	64	147.0 ± 3.8	8.6 ± 0.5	10.7
Chr. E	72	2954	184	22	40.6 ± 1.1	2.5 ± 0.14	11.5
Root tip							
Chr. E	37	1516	175	12	41.0 ± 0.6	4.7 ± 0.3	7.1

*The lengths given above for *T. erectum* microspore chromosomes have been converted from measurements made on 2BD-crystal violet preparations using the factor 1.6. All other measurements are from material fixed in alcohol-acetic or aceto-carmine.

**A = number of attachments; see text, p. 338.

In an earlier analysis, made primarily to determine direction of relational twists and therefore not including completely untwisted metaphase chromosomes (Fig. 18), similar data had been obtained from 72 complete *T. erectum* microspores fixed in 2BD. Lengths have been converted into aceto-carmine



TEXT-FIG. 2. Graph showing relationship between chromatid length and number of relational twists in chromosomes of microspores of *T. grandiflorum* (solid circles) and *T. erectum* (hollow circles). The large circles are means for the Groups I to IV of Table IV.

equivalents and the data divided into four groups of 18 each. The mean lengths of these correspond roughly to the mean group lengths in *T. grandiflorum*, with the exception, of course, of the fourth group whose mean is greater in *T. erectum*, since it did not include the latest untwisted metaphase stages. Individual microspores, in this species, ranged in total chromatid length from 269μ at mid-prophase to 94μ at the latest stage at which twists were still present. The number of twists ranged from 20 to 2. The mean length of the longest group was 194.1 ± 6.8 and of the shortest 117.3 ± 1.8 . The mean number of twists in these two groups is 11.8 ± 1.1 and 7.5 ± 0.9 .

From Table IV and Text-fig. 2 it is evident that the relationship between prophase contraction and loss of twists is different in the microspores of the two species. At early prophase the mean gyre length (of the relic coil) has been found to be approximately the same in both species (Table III). The initial frequency of twists per unit length should therefore be approximately the same in both if our conclusion that relational coiling derives from plectonemic coiling is correct. But at later prophase stages (Table IV, Groups 1 to 3) it is apparent that chromosomes of *T. erectum* have far fewer twists per unit length than have those of *T. grandiflorum*. It seems probable that the timing relationship between contraction, i.e., length, and untwisting differs somewhat in the two species. If length is used as a criterion of the stage of

TABLE V
THE DIRECTION OF TWISTING IN *E* CHROMOSOMES OF MICROSPORES AND ROOT TIPS IN *T. erectum* AND *T. grandiflorum*
(U = untwisted)

Material	Number of chromosomes	Group 1†	Group 2†	Group 3†	Group 4†
<i>T. erectum</i> Microspore	72	L.L.LLR L.LR R.R LL.R LR.RR U.R L.L U.L U.LRL LR.L U.LRLRR U.LRR LLL.LRR L.R R.R U.L U.RL L.RR	R.L R.LRR L.L LL.RRR L.R RL.R R.R R.U RR.LL L.R U.L R.L L.L R.L L.LL U.LL L.RL L.LL U.RL R.LL L.R	LL.U LL.L LL.L L.RR RL.LR L.RR U.L RL.LR U.LLL U.R L.L L.R U.L U.R L.R U.L L.LL R.L LR.L L.RR*R RRR.U LLL.LL RL.RRR RR.U R.LL L.RR	RL.U R.U R.U L.L RR.RRR R.L RR.R L.LLR U.L L.R RR.U LL.U U.L R.L L.R RR.RR L.L RR.RR LR.R RR.LLL R.LL U.LL RR.R RRL.RRR R.RRR L.LL RL.RR
Root tip	37	LLRR.LLLL RR.R RR.RR RRRR.RRRR RRR.R RR.RR RRRR.RRR RRR.LLLL LRRR.LLLL	LL*LL.L.LR LL.RR*R RR.LLL LRR.LLLL RL.RR*R LLL.RR RLL.RR L.RRR R.RR*R	LL*LL.L.LR LR.L L.RR*R RRR.U LLL.LL RL.RRR RR.U R.LL L.RR	RR.RR LR.R RR.LLL R.LL U.LL RR.R RRL.RRR R.RRR L.LL RL.RR

TABLE V—Concluded
THE DIRECTION OF TWISTING IN *E* CHROMOSOMES OF MICROSPORES AND ROOT TIPS IN *T. erectum* AND *T. grandiflorum*—Concluded
(U = untwisted)

Material	Number of chromosomes	Group 1†	Group 2†	Group 3†	Group 4†
<i>T. grandiflorum</i> Microspore	44	RRL . RRRRL LLR . LIRRL RRLL . LRRRL LLLLL . RRRRR RRR . LILRL RRLL . LLL RRRRL . RRRLL LL . LLLL L . RRL RRL . LLLL	RRRR . LLLR LLL . LL RRLRR . RRRR LLLL . LLL LLR . LLRRR LR . L RR . L LRR . LLR RR . RRR RL . LL	LR . LRLLL LLLL . RRR LL . LLR LLL . RRR RLL . RRL RLL . RRR L . LLL R . L LRR . RLL LLLL . LLR	L . RRRR L . R R . LL LL . RRR RR . LL U . R U . R U . U U . U U . U U . U
Root tip	50	R*R . RRR RR*R . RR LL . LL LL . L RRR . LL LR . U LL . RRR L . RRR LLL . RRL LLL*L . LRR L*LRRLL*LL . L*LRRRR LL . RRR	RLL . RRL R . L*LL RRR . RR*R L . RR RR . L RRR . L LL . LLL L . R R . L LLL . U RRR . RRL L . RL	L . U RR . LL LLLL . LL LLL . LL*L RR . L U . RL RR . LLL LLL . RRR*R LL . LLL L . R R*RRL . RRR U . RRR	R . R RR . L*LL*LL*L L . LLL U . RR RR . RRR LLLL . L RR*RRR . RRRR LL . RR LL*L . RR RR . LLL LLL . RRR RR*RL . LLL LLL . RL LL . LLL

† For microspores these refer to the same groups as in Table IV.

* A relic coil at the position indicated.

mitosis, it appears that untwisting occurs earlier in *T. erectum* and proceeds more quickly at later stages in *T. grandiflorum*.

The mean intertwist distances $\left(\frac{L}{T+A}\right)$ for whole complements have been calculated (Table IV) by dividing the chromatid length (L) by the number of relational twists (T) plus the number of attachments (A). The means for comparable groups are seen to be consistently lower in *T. grandiflorum* than in *T. erectum*, a further indication that untwisting takes place earlier in the latter.

The *E* chromosomes from root tip cells of *T. erectum* and *T. grandiflorum* have been analysed for comparison with microspore chromosomes (Tables IV and V). Partly on account of the double number of chromosomes in root tips (Fig. 29) it is much more difficult to obtain data on number and direction of twists from correspondingly early stages. There is consequently a much smaller range in chromatid length in the data presented. The range in number of relational twists, however, remains high and it is therefore difficult to determine a relationship between length and number of twists. Relic coils were observed in a large number of somatic chromosomes and, as in microspore relic coils, they were plectonemic in all cases. Positions where the relationally coiled chromatids still retain gyres of the relic coil are marked by an asterisk in Table V, which also gives the direction of twisting for all *E* chromosomes in both microspores and root tips in the two species. The most obvious difference between the relational coiling in root tip and in microspore chromosomes is the higher frequency of intrabrachial changes of direction in the latter (Table IX).

The Direction of Coiling

The direction of coiling in synaptic, desynaptic, and asynaptic *Trillium* has been discussed by Huskins and Wilson (20). Further studies on first and second anaphase and, in addition, on the relic spirals of first microspore division prophase of synaptic material have now been made and analysed as before. Table VI gives the mean number of gyres for a complement of five chromatids, the mean number of changes per cell, and the "effective" and true chiasma frequencies. "Effective" here refers to the effect that chiasmata have in permitting or causing changes of direction. If two chiasmata, or a chiasma and an attachment, are very close together (within one half-gyre length) they must act as a unit in this respect. The true chiasma frequency has been obtained from the analyses of camera lucida drawings of chromatids made by Dr. H. B. Newcombe. When chiasmata close together in these were counted as one, the frequency was almost identical with that obtained in rough counts made without drawing. The latter method was therefore used in collecting most of the data.

The data from Table VI on changes in direction have been analysed (in Table VII) in a manner somewhat similar to that used by Huskins and Wilson (20) in their Table VII. The following modifications have, however, been intro-

duced: (1) the effective, instead of the true, chiasma frequency has been used in calculating the number of changes assumed to be associated with chiasmata (Table VII, Column 4), (2) the subterminal attachment of *A* is omitted as it does not affect the number of changes, and (3) whole gyres have been used instead of half gyres.

TABLE VII

ANALYSIS OF CHANGES IN DIRECTION* OF THE MAJOR AND RELIC COILS OF *T. erectum*

Material	Mean number of intra-brachial changes per cell	Per cent gyres with intra-brachial changes	Per cent possible** changes realized	Number of changes assumed to be associated with chiasmata	Remaining number of changes	Per cent gyres with intrabrachial changes not associated with chiasmata
Major coils						
Synaptic						
58-2E-1	24.8	17.4	11.6	14.4	10.4	7.2
58-4E-1	29.4	17.9	11.0	17.6	11.8	7.2
58-5H-3	30.0	21.1	14.1	20.0	10.0	7.0
58-5d-3	37.8	20.6	12.9	17.5	20.3	11.0
58-5d-3(4II)	32.0	19.0	12.1	17.5	14.5	8.6
65-L2-a	38.0	16.0	9.1	13.3	24.7	10.5
H.&S. (19) and Hunter (16)	34.6	15.0	8.8	17.2	17.4	7.5
Mean		18.1	11.4			8.4
Desynaptic***	24.5	18.5	12.7	10-17	14 5-17 5	11.0-5 8
Asynaptic***						
Type 1	17.0	6.0	3.4	0.0	17.0	6.0
Type 2	34.0	8.1	4.5	0.0	34.0	8.1
Mean		7.05	3.45			7.05
Microspore						
prophase relics						
66-T-117	29.6	19.4	12.7	17.0	12.6	8.3
66-T-72	38.0	16.2	9.6	17.2	20.8	8.1
Mean		17.8	11.15			8.2

*Calculations based on the number of intrabrachial changes only, i.e., an average of eight less (owing to the attachments) than the total number of changes observed for the whole complement (Table VI).

**See text, p. 344, for method of calculation.

***Huskins and Wilson (20).

Knowing the effective chiasma frequency, and assuming that the direction of coiling is random on either side of chiasmata and attachments, as indicated by the data of Huskins and Wilson (20), the number of changes associated with each can be estimated. Considering only intrabrachial changes (Table VII, Column 1), i.e., eliminating interbrachial changes (those at the attachment), the percentage of gyres with changes (Table VII, Column 2) varies considerably between different materials. It reaches a mean of 18% in

normal synaptic material, but one of only 7% in asynaptic. The means for the same two materials are 8.4 and 7% respectively when only those intrabrachial changes not associated with chiasmata are considered (Table VII, Columns 5 and 6). From this it seems apparent that intrabrachial changes not associated with chiasmata are approximately proportional to gyre number and that the more extreme variations in frequency of changes are directly related to chiasma frequency.

Changes of direction in the relic coil (Figs. 10, 13, 19, 20) have been analysed for comparison with changes at first meiotic anaphase (Table VII). The mean percentages of gyres with intrabrachial changes are 17.8% for relic and 18.1% for the major coil. It has been previously shown that the mean number of gyres at early microspore prophase is only slightly less than at second anaphase (Table III, 66–72). These data provide direct evidence that the coils of microspore prophase are really relics of the major coil of meiosis, as Darlington's name for them implies.

TABLE VIII

DIRECTION OF RELATIONAL TWISTS AT ATTACHMENT REGIONS IN MICROSPORE CHROMOSOMES AND IN ROOT TIP *E* CHROMOSOMES OF *T. grandiflorum* AND *T. erectum*

—		R.R	R.L or L.R	L.L	R.U or U.R	L.U or U.L	U.U
Microspores							
<i>T. grandiflorum</i>	<i>B</i>	16	7	10	2	4	5
	<i>C</i>	9	9	7	5	8	6
	<i>D</i>	8	11	8	9	1	7
	<i>E</i>	4	22	12	1	1	4
	Total	37	49	37	17	14	22
<i>T. erectum</i>	<i>B</i>	5	1	4	35	14	14
	<i>C</i>	6	11	7	20	17	12
	<i>D</i>	9	12	5	17	14	16
	<i>E</i>	9	25	15	8	15	0
	Total	29	49	31	80	60	42
Root tips							
<i>T. grandiflorum</i>	<i>E</i>	7	25	12	4	2	0
<i>T. erectum</i>	<i>E</i>	12	19	3	2	1	0

The direction of relational twisting has been analysed in order to determine, first, if the direction of coiling is at random across the attachment (Table VIII) and, secondly, whether there is any significant deviation from randomness in the total numbers of right and left twists (Table IX). If there were no inherent tendency for a chromosome or a chromosome arm to coil more in one direction than the other in those chromosomes without changes at the attachment, the number of R.R's should be equal to the number of L.L's.

TABLE IX

NUMBERS OF RIGHT AND LEFT RELATIONAL TWISTS IN MICROSPORE AND ROOT TIP CHROMOSOMES OF *T. grandiflorum* AND *T. erectum*

	A		B		C		D		E		Total	
	R	L	R	L	R	L	R	L	R	L	R	L
Microspores												
<i>T. grandiflorum</i>	54	69	74	81	55	61	85	89	121	145	389	445
<i>T. erectum</i>	61	45	68	44	50	58	66	51	86	95	331	293
	A to D											
	R				L							
Root tips												
<i>T. grandiflorum</i>									124	127	196	182
<i>T. erectum</i>									105	68	167	120

If coiling were random across the attachment we should expect the number of chromosomes with their proximal twists in the same direction (R.R and L.L) to be equal to the number which have a change of direction (R.L and L.R) at the attachment. In both species *B* chromosomes without changes are in excess but the numbers, in *T. erectum*, are too small to be reliable. It may be significant that the *B* chromosome has a submedian attachment whereas *C*, *D*, and *E*, which tend towards equality, have more nearly median attachments. The attachment therefore appears to be a point of random change in chromosomes *C*, *D*, and *E* but possibly not in *B*. In chromosomes without changes at the attachment the observed numbers of R.R and L.L deviated significantly from equality in only one instance, the root tip *E* chromosome of *T. erectum*. However, the numbers are too small to be very reliable and when the root tip *E* chromosomes of both species are considered together, the numbers fit a 1 : 1 ratio. So far only twists proximal to the attachments have been considered, but when all twists are considered (Table IX) there is still a random distribution of right and left twists in all but two of the 14 totals. In *T. erectum* the *B* chromosome of the microspores and the *E* chromosome of the root tips have an excess of right-handed twists.

An analysis of intrabrachial changes of direction in relational twisting has been made in microspore and root tip chromosomes of both species. Direction of twisting in the *E* chromosome has been given in Table V, and the frequency of changes, when two or more twists are present in an arm, is given in Table X as the mean number of twists for each change of direction. The latter table shows that the frequency of reversals in microspore chromosomes is more than twice that of the root tip *E* chromosome.

Frequencies of intrabrachial changes of direction have been obtained from both the major and relational coils (Table VII and XI). In order to compare such frequencies it seems advisable to express both in the same terms by transposing the gyres of the major coil into their equivalent number of re-

ional twists. In a plectonemic coil an arm of n gyres will, in the absence of unwinding, give a relational coil of $2n - 1$ relational twists if no changes of direction are present. Each change of direction in the plectonemic coil will reduce by one the equivalent number of relational twists, i.e., a major coil will give a maximum number of relational twists when no changes of direction are present.

TABLE X

FREQUENCY OF INTRABRACHIAL CHANGES OF DIRECTION IN MICROSPORE AND ROOT TIP CHROMOSOMES

—	Number of relational twists*	Number of intrabrachial changes	Mean number of twists per change
Microspores			
<i>T. grandiflorum</i>			
<i>E</i> chromosome	254	38	6.7
All chromosomes	750	104	7.2
<i>T. erectum</i>			
<i>E</i> chromosome	104	21	4.8
All chromosomes	344	56	6.2
Root tips			
<i>T. grandiflorum</i>			
<i>E</i> chromosome	233	15	15.5
<i>T. erectum</i>			
<i>E</i> chromosome	162	12	13.5

*Considering only arms with two or more twists.

TABLE XI

ANALYSIS OF INTRABRACHIAL CHANGES OF DIRECTION IN MICROSPORE CHROMOSOME ARMS HAVING TWO OR MORE RELATIONAL TWISTS

Species	Number of twists per arm	Number of arms	Number of arms with:					Total number of changes	Mean number of twists per change	Percentage of possible changes realized
			All R's	All L's	One change	Two changes	Three changes			
<i>T. grandiflorum</i>	2	89	34	34	21	0	0	21	8.5	24
	3	62	16	17	27	2	0	31	6.0	25
	4	31	7	8	13	3	0	19	6.5	20
	5	25	3	7	11	4	0	19	6.6	19
	6-7	14	3	1	6	4	0	14	6.1	18
	8-11	5	2	0	0	3	0	6	7.7	15
<i>T. erectum</i>	2	115	44	37	34	0	0	34	6.8	30
	3	26	7	8	8	3	0	14	5.6	27
	4	9	3	2	2	0	2	8	4.5	30

The number of possible changes of direction in one arm of a relational coil is one less than the number of twists. Therefore in an arm with $2n - 1$ relational twists, there will be $2n - 2$ different pairs of adjacent twists and for a complement of a arms $2n - 2a$ where n is the total number of gyres in each case. As the number of changes in direction cannot exceed the number of pairs of adjacent twists the number of changes can be expressed as a percentage of the total number of pairs, or, in other words, as the percentage of possible changes realized. In Tables VII and XII changes in the major and relic coils have been expressed as a percentage of changes in a relational coil, or coils, whose maximum number of possible changes is $2n - 2a$ (this is, of course, the number of adjacent pairs of twists). The values obtained (Table VII, Column 3, and Table XII, right-hand column) can be compared directly with the percentage of possible changes actually realized (Table XI) in the relational coils studied at microspore prophase.

TABLE XII

INTRABRACHIAL CHANGES OF DIRECTION IN ARMS WITH 2 TO 11 GYRES, FROM FIRST ANAPHASE OF *T. erectum* (66-T-81-c)

Number of gyres per arm	Number of arms	Total number of gyres	Total number of changes	Calculated number of possible changes	Percentage of possible changes realized
2	29	58	2	58	3.5
3	38	114	8	152	5.3
4	64	256	39	384	10.2
5	36	180	29	288	10.4
6	24	204	26	240	10.8
7	50	350	71	600	11.8
8	59	472	84	826	10.2
9	41	369	72	656	11.0
10	9	90	19	162	11.7
11	3	33	6	60	10.0
Total	353	2126	356	3426	Mean = 10.4

In Table XI the frequency of interstitial changes observed in the relational coils has been analysed by grouping all chromosome arms that have the same number of twists. The *T. erectum* data comprise only arms with 2, 3, or 4 twists. In these an average of 29% of the possible total number of changes is realized. In *T. grandiflorum* as many as 11 twists were found in one arm. In those with 2, 3, and 4 twists 23% of the possible number of changes is realized. In arms with 8 to 11 twists, only 15% is realized—for the complete range see Table XI. The decrease for each extra twist is small but the trend is constant and it is steadily approaching the percentage found in the major or relic coil of *T. erectum* (approximately 11%, Tables VII and XII). It therefore seems probable that, if sufficient observations could be made at earlier stages in the development of the relational coil before untwisting occurs, it might reach the same figure.

A similar analysis of intrabrachial changes in direction in the major coil has been made by grouping together all arms that have the same number of gyres and calculating the "percentage of possible changes realized" for each group (Table XII). The percentage realized increases from 3.5 to 10.2 as the number of gyres increases from two to four, but there is no appreciable further increase in arms with more than four gyres. It seems apparent, therefore, that in the relational coil the increase in the percentage of possible changes realized in arms with a small number of twists (Table XI) cannot be attributed to a higher frequency of major coil changes in the shorter arms. Chronologically, during prophase, the frequency of changes in the relational coil increases as the number of twists decreases. This must be taken to mean that a larger proportion of twists than changes are being eliminated. The obvious interpretation is, that during prophase, untwisting or cancellation of relational twists takes place at intrabrachial changes of direction. This occurs when wire models of plectonemic coils are drawn out into relational coils.

Discussion

The Major Spiral

Meiotic prophase contractions prior to the formation of the major coil have been measured by several workers. These have been summarized by Darlington and Upcott (10, Table I) and will not be repeated here. Relatively little attention, however, has been given to elongation, although Belar (1) suggested in 1928 that it might be related to spiral formation. Wilson and Huskins (50) demonstrated chromonema elongation during the formation of the major coil. They reported the chromonema to elongate to at least twice its former length (at diakinesis) whereas additional measurements by the present authors show that the increase between diakinesis and first anaphase¹ is probably not less than threefold.

In comparisons of chromosome lengths made between individual complements of any one plant only 75% are shorter at first anaphase than at diakinesis. The mean anaphase lengths, however, are consistently shorter, which indicates that a slight contraction of the matrix may occur in *Trillium*. It cannot compare in magnitude with that found in *Tradescantia* by Sax and Humphrey (41) and Wilson (unpublished).

Further analyses of changes of direction of the major coil at first anaphase give results in complete agreement with those of Huskins and Wilson (20) who conclude that chiasmata and the attachment are points of random change and that in addition there occur a number of changes that are proportional to the number of gyres. The frequency of changes of direction in a limited number of second anaphase and microspore relic coils agrees very well with the frequency at first anaphase. Direct evidence is therefore provided that in *Trillium* the microspore prophase spiral actually is a relic of the major coil

¹ See footnote in Wilson and Huskins (50, p. 268) for discussion regarding the nature of this elongation.

of meiosis. In forms having an extended interkinesis it is presumably a relic of the standard coil of the second division.

The direction of coiling of the major spiral of *T. kamtschaticum* has been analysed by Matsuura (32, 33). He concluded (33) that the number of changes is a function of the length of the chromosome arm (the equivalent of Huskins and Wilson's third factor) and that their origin is apparently fortuitous and bears no relationship to chiasmata. It may be pointed out, however, that this species of *Trillium* has a very low chiasma frequency. Changes associated with chiasmata would therefore be relatively few and hence would not significantly alter the expectation based on length. The authors have analysed the data from Matsuura's (32) Table III for the position of changes in what they call the *E* chromosome (his *A*) and have found the frequency significantly greater between the third and fourth and between the fourth and fifth gyres (from the attachment) than in any other region. There is frequently a chiasma at approximately each of these positions in both arms of the bivalent.

The data from the major and relational coils are incompatible with hypotheses, such as those of Darlington (5) and MacKnight (29), that assume coiling to be produced by an internal torsion with its direction controlled from the centromere or attachment (though it is possible that the position of the attachment may affect relative direction of coiling in the two arms). That an explanation of coiling based on this assumption runs into serious difficulties has already been recognized by Darlington (8, p. 491). The possibility of a spiral molecular structure within the chromonema remains, however, and Nebel's (35) hypothesis, which is in some respects similar, but allows for intrabrachial changes of direction, cannot entirely be ruled out by the data presented here. However, the association that has been found between chiasmata and changes of direction very greatly reduces its plausibility, or else demands important extension and modification of it.

As a partial and provisional hypothesis, Wilson and Huskins (50) suggest that coiling results from elongation taking place within a confined space bounded by the pellicle. The chief function of the pellicle may be to regulate the structure of the spiral and ensure the evenness of its diameter; that it is intimately related to spiralization is indicated by the observation that in material that failed to develop matrix and pellicle as a result of high temperature a mere waviness replaced the usual spiral (Figs. 6 and 7; also Wilson and Huskins, 50). The primary requirements of such an hypothesis of coiling would be (a) the existence of a pellicle, (b) elongation of the enclosed chromonema, and (c) a resiliency sufficient to prevent the chromonema from folding instead of coiling. There is now evidence for all of these requirements. Before attempting to formulate any more definite hypothesis of coiling however, it is desirable to have more data on the somatic coil of microspore and root tip chromosomes. If an hypothesis consistent with a wide range of well established microscopic data can be formulated, the way will then be clear for combining this with biophysical and microchemical data. Such a

combination will allow incursions, more well founded than have so far been possible, into the problem of the submicroscopic factors which underlie the visible.

Relational Coiling

Since the chromatids which are relationally coiled at microspore prophase are the product of the tertiary split of meiosis (Figs. 5, 8) it is obvious that the arrangement, in the major and relic coils, of the resultant half-chromatids must bear an intimate relationship to the mechanism of relational coiling. Text-fig. 1A illustrates diagrammatically how a relational coil can result from drawing out the gyres of a plectonemic coil. If it is assumed that the relational coil at microspore prophase originates in a similar manner from a plectonemic relic coil it is apparent that relational twists would not compensate for the relic coils, except across changes in direction. In accord with this assumption was the complete absence of compensating relational twists in arms or regions devoid of changes in direction. These observations do not agree with Darlington's (5) assumption that relational twists are compensatory. He observes that "relational spirals can sometimes be seen in the same direction as relic spirals" and he, therefore, has to ascribe non-compensating relational twists to a secondary slipping of the chromatids around one another while the relic coil is still present. This assumption does not appear to be based on observations: "the relic spirals *imply* [the authors' italics] the existence of a compensating relational spiral of chromatids in the opposite direction". In any case this explanation is not valid for *Trillium* because the tertiary split, sometimes visible in the major coil, results in a plectonemic spiral. If compensating relational twists are present at a later stage *they* must be developed secondarily and not the non-compensating type as Darlington suggests.

Upcott (47) has made a study of relational and relic coiling in *Hyacinthus* and found 95 nucleolar chromosomes "with coils of recognizable direction. With three exceptions, these showed no change of direction of relic coiling within a chromosome arm". Later these three exceptions are ignored and she states that "the fact that there is no change of direction in the relic coiling except at the centromere implies that at the previous division, which was meiosis, the internal coiling from which these coils are presumed to be derived is consistent in direction". Changes in direction of the relational coil she attributes to there being two kinds of relational coiling, the first, she assumes, compensates for the relic and is in the opposite direction, whereas "true relational coiling" is brought about by the development of the new internal coil. This is a restatement of Darlington's (5) wholly deductive hypothesis. It would seem to us much simpler to consider relational coiling to be of only one type, whose origin may be traced directly to the plectonemic relic coil, and hence back to the major coil of meiosis. Intrabrachial changes of direction being present in the antecedent coils it should, on this basis, be expected that there would be changes of direction in the relational coil, and, therefore, no great significance can be attached to the fact that the relational

coiling of one region within a chromosome arm may be in the opposite direction to relic coils of another region. Neither can we agree with Upcott that the direction of coiling in one division has an indeterminate relationship with that of the next.

With the possible exception of the microspore *B* chromosome the direction of relational coiling is random across the attachment in both *T. erectum* and *T. grandiflorum* as reported for the major coil of *T. kamtschaticum* by Matsuura (31, 33). There is no obvious explanation for the apparently anomalous behaviour of the *B* chromosome, but it may be associated with the non-median position of the attachment. It seems not unlikely that in chromosomes with arms of very different length the direction of coiling in one arm may affect the direction of coiling in the other. Unfortunately, this possibility cannot be tested in the present materials, since chromosomes *C*, *D*, and *E* have their attachments nearer the median position and in the *A* chromosome it is so nearly terminal that the direction of coiling cannot be determined in the short arm. Further work is required to settle the problem.

The proportion of intrabrachial changes of direction in the relational coil is higher than in the relic (or major) coil when changes in both are expressed in the same terms, i.e., as the percentage of possible changes realized. The percentage in the relational coil does not, however, appear to be stable, though this has not been established statistically. It appears to increase with a decrease in the number of twists and in arms with the greatest number of twists it approaches the percentage found in the major coil. When a model plectonemic spiral with changes of direction is pulled out into a relational coil it unwinds from the changes and thus increases the number of changes relative to the number of twists. This seems to provide the obvious explanation of the difference in frequency of intrabrachial changes between the relational and relic coils.

The frequency of intrabrachial changes of direction in the relational coils of root tip chromosomes is about one-half that of microspore chromosomes. If changes in the latter are sequential to those in the major coil, some of them must be due to chiasmata. Since this factor would be inoperative in root tip cells the frequency of changes of direction in their chromosomes would, other things being equal, be expected to be less than in microspores. The difference in diameter between the somatic and major coils may, however, contribute to the difference in frequency of changes in direction.

The relational coil of the first microspore division in *Trillium* is clearly preceded by a plectonemic coil from which it is apparently derived. The microspore relic coils of *T. undulatum*, *Aloe*, *Gasteria*, and *Tradescantia* have also been found to be plectonemic in all of a relatively small number examined. Although preparations have not yet been obtained in which the somatic coil can be analysed directly, wherever the two chromatids of somatic prophase relic coils can be distinctly observed in gyres of regular spiral form their relationship is plectonemic. Further, White (49) illustrates plectonemic

the coils in spermatogonial prophases of certain Orthoptera. It seems not unlikely therefore that prophase *chromatid* relational coiling is, in general, preceded by and derived from a plectonemic spiral.

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CHROMOSOME BEHAVIOUR IN F_1 WHEAT HYBRIDS

I. PENTAPLOIDS¹

BY R. MERTON LOVE²

Abstract

Meiosis was studied in varieties of *Triticum vulgare* ($2n = 42$), *T. dicoccum* ($2n = 28$), *T. durum* ($2n = 28$), *T. Timopheevi* ($2n = 28$), and in 16 of their pentaploid hybrids as part of a study in an attempt to establish criteria indicating relationships between 42- and 28-chromosome wheats, with particular reference to the possible relationship of the new 42-chromosome wheat, McMurachy's Selection, to *T. dicoccum* or *T. durum*.

One plant each of *T. vulgare* var. Hope and Marquillo had only 41 chromosomes. One plant of *T. durum* var. Pentad had three times as many unpaired chromosomes as the other plants of this variety.

A nucleus with 14 pairs and 7 univalents was not detected among the 86 pollen mother cells analysed in the cross involving *T. Timopheevi*. In the remaining crosses the frequency of this association of chromosomes was lowest in the three hybrids involving *T. durum* var. Pentad, greater in the three involving *T. dicoccum* var. Khapli, still greater in the three involving *T. dicoccum* var. Vernal, and greatest in the nine hybrids involving *T. durum* var. Iumillo.

Of the seven "extra chromosomes" of *T. vulgare* only six remained unpaired in some pollen mother cells of the hybrids involving Vernal or Iumillo and five in those involving Khapli or Pentad. One pollen mother cell of F_1 Marquis \times Pentad contained only four unpaired chromosomes.

Associations of four chromosomes were rare in some, and not seen at all in others, of the hybrids involving Vernal or Iumillo, more frequent in hybrids involving Khapli, and very frequent in hybrids involving Pentad. In the latter, from 47 to 57% of the nuclei had from one to three such multiple associations, and even chains of five and six chromosomes were observed.

Fragmentation of unpaired chromosomes at or in the spindle fibre attachment region was observed in a number of first anaphase figures.

There were statistically significant differences in the frequencies of occurrence of micronuclei in tetrads of the 15 hybrids studied at the second reduction division.

The crosses R.L. 1544 (genetically related to *T. durum* var. Iumillo) \times Iumillo and Hope (genetically related to *T. dicoccum* var. Vernal) \times Vernal were used as standards for comparison. On the basis of the results, the following criteria were used in attempting to establish relationships between the other 42- and 28-chromosome wheats: (1) the percentage of pollen mother cells with 14 pairs and 7 univalents (greatest in the hybrids between related varieties); (2) the average number of chromosomes involved in multiple associations (lowest in hybrids between related varieties); (3) fertility (greatest in hybrids between related varieties). McMurachy's Selection appeared to be most closely related to *T. durum* var. Iumillo. On the basis of Criteria (1) and (2), Marquis appears to be more closely related to *T. dicoccum* var. Vernal than to *T. durum* var. Iumillo, but in respect of fertility it seems closer to the latter.

Chromosome behaviour in the 16 hybrids cannot be neatly summarized. Even varieties within a species gave different results—results that are not in agreement with earlier published reports on chromosome behaviour in pentaploid wheat hybrids in which it has been stated that 14 bivalents and 7 univalents are most commonly found. The difficulties encountered in attempting to establish criteria indicating relationships between the 42- and 28-chromosome wheats suggest that the utmost caution must be used in drawing phylogenetic conclusions on the basis of such data.

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Introduction

Cytological studies conducted by the writer (16, 18, 21) on *Vulgare*-like derivatives of the crosses *Triticum vulgare* var. R.L. 729, Hope, Marquillo, and Marquis \times *T. durum* var. Iumillo have shown many plants of the fifth to seventh generations to be heterozygous for differences in the arrangement of chromosome segments. This was evidenced by the presence of inversion bridges and accompanying fragments, associations of three and four chromosomes (the latter forming chains or rings), chromosomes deficient for one complete arm, and chromosomes with a deficiency of one arm and a duplication of the other. All these types of aberration were transmitted from parent to progeny in some instances. Since the results referred to above could hardly be expected on the basis of previously reported accounts on the behaviour of chromosomes in similar hybrids (cf. the review by Aase (1)), these crosses were repeated and the F_1 plants examined in detail cytologically.

There was also studied a series of crosses in an attempt to obtain evidence concerning the species involved in the origin of the variety McMurachy's Selection ($2n = 42$). This variety was recently found in a field of *Triticum vulgare* var. Garnet in Western Canada (20). It is unusual in that it is virtually immune to wheat stem rust under field conditions in Western Canada, but it resembles Garnet wheat in some morphological characters, in its breeding behaviour, and also in baking quality. It seems probable that this strain resulted from a natural cross between Garnet and some other wheat variety possessing stem rust resistance. This unknown parent may have been a variety of *T. vulgare*, *T. dicoccum*, or *T. durum*. The results of the interspecific crosses relating to the problem are reported in this paper.

Studies are being conducted at the Cereal Division, Ottawa, involving the 28-chromosome wheat, *T. Timopheevi* Zhuk. At the request of Mr. J. G. C. Fraser, the hybrid between *T. vulgare* var. Canus and *T. Timopheevi* was examined cytologically. Cytological data on this hybrid, though few, are presented for comparison with those obtained on the other hybrids.

Materials and Methods

PARENTS

Parental varieties were selected to represent the species that might have been involved in the natural cross from which McMurachy's Selection was derived, namely, *Triticum dicoccum* Schülb., *T. durum* Desf., and *T. vulgare* Host. These were as outlined below.

Vernal (R.L. 216)* and Khapli (R.L. 653) representing *T. dicoccum*.

Iumillo (R.L. 7) and Pentad (R.L. 203) representing *T. durum*.

* Dominion Rust Laboratory Accession Number. The origin of the selections of the varieties used is given here since it is possible that the same crosses with other selections might give different results.

*¹ Marquis (R.L. 84) representing varieties of *T. vulgare* that have not been derived from crosses with the 28-chromosome wheats. It was derived from the intraspecific hybrid Hard Red Calcutta \times Red Fife (2, 19).

Hope (R.L. 209) representing varieties of *T. vulgare* derived from crosses with *T. dicoccum*. It was derived from the hybrid Marquis \times Yaroslav (Vernal) Emmer (5).

R.L. 1544, a selection from the cross Marquis \times Iumillo (21), representing varieties of *T. vulgare* derived from crosses with *T. durum*.

McMurachy's Selection (R.L. 1313) was of course also used.

In addition, the following varieties of *T. vulgare* were used as parents in crosses with Iumillo: Marquillo (R.L. 1943), derived from a Marquis \times Iumillo cross (2), and R.L. 729, a Dominion Rust Laboratory selection from the cross Marquis \times Pentad. Canus was crossed with *T. Timopheevi*. The former was not analysed cytologically and only one plant of the latter was examined.

HYBRIDS

T. vulgare \times *T. dicoccum*

- | | |
|------------------------------|------------------------------|
| 1. Hope \times Vernal | 4. Hope \times Khapli |
| 2. Marquis \times Vernal | 5. Marquis \times Khapli |
| 3. McMurachy \times Vernal | 6. McMurachy \times Khapli |

Hope is related to *T. dicoccum* and Marquis is not. The crosses involving these two varieties were intended to serve as standards with which to compare the crosses involving McMurachy.

T. vulgare \times *T. durum*

- | | |
|-------------------------------|-------------------------------|
| 7. R.L. 1544 \times Iumillo | 10. R.L. 1544 \times Pentad |
| 8. Marquis \times Iumillo | 11. Marquis \times Pentad |
| 9. McMurachy \times Iumillo | 12. McMurachy \times Pentad |

In this series, R.L. 1544 is related to *T. durum* and Marquis is not. The crosses involving these two varieties were intended to serve as checks.

In addition, the following hybrids were studied:

- | | |
|--------------------------------|---|
| 13. Marquillo \times Iumillo | 15. Iumillo \times Hope |
| 14. Iumillo \times R.L. 729 | 16. Canus \times <i>T. Timopheevi</i> |

All crosses, except No. 16, were made by Dr. R. F. Peterson at the Dominion Rust Research Laboratory, Winnipeg, Man. Cross No. 16 was made by Mr. F. Gfeller at the Cereal Division, Ottawa. The F_1 hybrids and the parents were grown in pots in the greenhouses of the Cereal Division, Central Experimental Farm, Ottawa, during the winter 1938-1939.

The data on fertility of the parents and hybrids were kindly supplied by Dr. R. F. Peterson. The fertility is given, for 5 to 20 plants of each parent and hybrid, as the percentage of first and second florets in the spikelets that set seed. It is to be noted that the fertility data were not recorded for the plants that were studied cytologically.

The observations of the first meiotic division were made from iron-acetocarmine smear slides made permanent by the method previously outlined by the writer (18). Temporary smears were used for the studies on material showing the second division. At least 100 first metaphase nuclei were analysed in each of the parent varieties and hybrids. In addition, the behaviour of paired and unpaired chromosomes at first anaphase was noted in many pollen mother cells. For the second division studies of the parents and of 54 of the 71 hybrid plants, 200 pollen mother cells (100 in each of two anthers) were examined in each plant and, for each cell, the numbers of micronuclei and inversion bridges were recorded.

Observations

PARENTS

In the 41 plants of the 11 parent varieties studied, 1928 complete first metaphase nuclei were analysed. The results of the analysis are summarized in Table I which includes the data on the second division observations and on fertility.

TABLE I
CHROMOSOME BEHAVIOUR AND FERTILITY IN THE PARENTS

Variety	2n	Number of plants	Number of first metaphase nuclei	Mean number of univalents and bivalents per PMC		Tetrads with micronuclei, %	Fertile florets, %
				I	II		
<i>T. dicoccum</i>							
Vernal	28	4	200	0 02	13 99	2 5	78 8
Khapli	28	4	118	0 28	13 86	3 4	82 9
<i>T. durum</i>							
Iumillo	28	4	237	0 12	13 94	1 6	86 5
Pentad	28	3	174	0 12	13 94	1 5	88 8
	28	1	58	0 36	13 82	?	
<i>T. vulgare</i>							
Marquis	42	4	263	0 18	20 91	4 0	85 3
Hope	42	3	166	0 26	20 87	5 3	89 7
	41	1	50	1 28	19 86	36 0	
Marquillo	42	3	100	0 26	20 87	8 4	?
	41	1	50	1 22	19 89	23 0	
R.L. 1544	42	4	130	0 08	20 96	3 1	86 0
R.L. 729	42	4	192	0 04	20 98	2 5	?
McMurachy	42	4	150	0 16	20 92	6 3	81 6

Although multiple associations of chromosomes have been recorded in varieties of *T. vulgare* by the writer (unpublished data) and others (7, 8, 9, 10) none were found in individuals of this species included in the present study. No multiple associations were found in the plants of *T. dicoccum* or *T. durum*. A chain of three chromosomes and a chain of four chromosomes, respectively, were seen in one and three of the 40 pollen mother cells examined in the one plant of *T. Timopheevi*. Evidences of inversions were detected in two Hope plants and have been reported by the writer (18) in other plants of R.L. 729.

One plant of Hope and one of Marquillo had only 41 chromosomes. One Pentad plant was characterized by the presence of an average of three times as many univalents as were found in the other three plants of this variety.

All plants, except one of Pentad and the one of *T. Timopheevi*, were included in the second division studies.

HYBRIDS

(a) First Division

(i) Associations of Chromosomes

In the 71 plants of the 16 hybrids, 6151 first metaphase nuclei were analysed. The data are summarized in Table II. It is seen from the data that there is little difference between the hybrids in the mean number of unpaired chromosomes—the hybrid Canus \times *T. Timopheevi* is one exception in this respect. There are differences, however, in the mean numbers of pairs of chromosomes. In most hybrids these differences are accounted for by the numbers of chromosomes forming multiple associations. This is best seen in the last column of Table II. Compare, for example, the hybrids R.L. 1544 \times Pentad and Marquis \times Pentad: the mean number of univalents is 7.69 and 7.61, respectively; of bivalents, 11.86 and 10.77, respectively; of chromosomes involved in multiple associations, 3.59 and 5.85, respectively.

It must be noted that there is much variability in chromosome pairing in the nuclei of all the hybrids. This is evident from the data in Table III, in which are recorded the maximum and minimum numbers of univalents, bivalents, and multiple associations of chromosomes found in the pollen mother cells of the 16 hybrids.

Tables IV and V illustrate some of the different ways in which the 35 chromosomes in the hybrids are arranged at first metaphase. Earlier published reports (cf. Aase (1) and Hector (6)) stated that 14 pairs and 7 univalents is the usual arrangement in pentaploid wheat hybrids. That particular arrangement was found in all hybrids studied, except the one involving *T. Timopheevi*, but the frequency of nuclei with such an arrangement varied from 4.5% in F_1 Marquis \times Pentad to 91.3% in F_1 Marquillo \times Iumillo (Table V). The arrangement, one association of 3 chromosomes, 13 pairs, and 6 univalents (Fig. 1) also occurs in all the hybrids, the frequency varying from 1.4% in F_1 Marquis \times Pentad to 13.3% in F_1 Iumillo \times R.L. 729. Since individual chromosomes cannot be identified, it is impossible to state that the same chromosomes are involved in any given type of association that occurs in a number of pollen mother cells. In the hybrid Iumillo \times R.L. 729, six is the lowest number of chromosomes observed unpaired in any one pollen mother cell, but it does not necessarily follow that it is always the same six chromosomes that remain unpaired. In those nuclei with only six unpaired chromosomes it is most probable that one of the so-called "extra chromosomes" derived from the third set of the hexaploid parent is associated with a pair derived from either the first or second set of each parent. It would be possible to have one of the extra chromosomes forming a bivalent with one

TABLE II
SUMMARY OF MEIOTIC CHROMOSOMAL ASSOCIATIONS IN F_1 PENTAPLOID WHEAT HYBRIDS

Hybrid	Number of plants	Number of PMC	Mean number* per PMC of associations of:						Mean number per PMC of chromosomes involved in multiple associations
			I	II	III	IV	V	VI	
<i>T. vulgare</i> × <i>dicoccum</i>									
1. Hope × Vernal	5	544	7 798	13 555	0 028	0 002	—	—	0 092
2. Marquis × Vernal	6	480	7 710	13 565	0 048	0 004	—	—	0 160
3. McMurachy × Vernal	2	231	7 951	13 117	0 173	0 074	—	—	0 815
4. Hope × Khapli	5	541	7 685	13 314	0 165	0 048	—	—	0 687
5. Marquis × Khapli	5	326	8 277	13 144	0 117	0 021	—	—	0 435
6. McMurachy × Khapli	4	200	8 035	12 860	0 260	0 110	0 005	—	1 245
<i>T. vulgare</i> × <i>durum</i>									
7. R.L. 1544 × Iumillo	6	395	7 340	13 701	0 086	—	—	—	0 258
8. Marquis × Iumillo	6	561	7 361	13 565	0 167	0 002	—	—	0 509
9. McMurachy × Iumillo	4	259	8 032	13 278	0 112	0 019	—	—	0 412
10. R.L. 1544 × Pentad	6	542	7 694	11 856	0 286	0 679	0 004	—	3 594
11. Marquis × Pentad	6	706	7 608	10 773	0 367	1 139	0 021	0 014	5 846
12. McMurachy × Pentad	3	313	7 493	11 930	0 335	0 639	0 010	0 006	3 647
13. Marquillo × Iumillo	5	402	7 123	13 898	0 027	—	—	—	0 081
14. Iumillo × R.L. 729	2	165	7 352	13 569	0 170	—	—	—	0 510
15. Iumillo × Hope	5	400	7 350	13 625	0 120	0 010	—	—	0 400
<i>T. vulgare</i> × <i>Timopheevi</i>									
16. Canus × Timopheevi	1	86	9 301	9 535	1 930	0 163	0 023	0 012	6 629

* Compare these with maximum and minimum numbers given in Table III.

TABLE III
MAXIMUM AND MINIMUM NUMBERS OF UNIVALENTS, BIVALENTS, AND HIGHER ASSOCIATIONS OF CHROMOSOMES FOUND IN NUCLEI
OF THE 16 HYBRIDS*

Hybrid	I		II		III		IV		V		VI	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
<i>T. vulgare</i> × <i>dicoccum</i>												
1. Hope × Vernal	17	6	14	9	1	0	1	0	0	0	0	0
2. Marquis × Vernal	15	6	14	10	1	0	1	0	0	0	0	0
3. McMurachy × Vernal	15	5	15	8	2	0	1	0	0	0	0	0
4. Hope × Khapli	15	5	15	0	2	0	1	0	0	0	0	0
5. Marquis × Khapli	17	5	14	9	2	0	1	0	0	0	0	0
6. McMurachy × Khapli	17	5	15	9	2	0	1	0	1	0	0	0
<i>T. vulgare</i> × <i>derum</i>												
7. R.L. 1544 × Iumillo	12	5	14	10	2	0	0	0	0	0	0	0
8. Marquis × Iumillo	13	5	14	11	2	0	1	0	0	0	0	0
9. McMurachy × Iumillo	14	6	14	9	1	0	1	0	0	0	0	0
10. R. L. 1544 × Pentad	16	5	14	6	2	0	1	0	1	0	0	0
11. Marquis × Pentad	14	4	14	7	4	0	3	0	1	0	1	0
12. McMurachy × Pentad	13	5	14	9	2	0	2	0	1	0	1	0
13. Marquillo × Iumillo	13	6	14	11	1	0	0	0	0	0	0	0
14. Iumillo × R.L. 729	11	5	15	12	1	0	0	0	0	0	0	0
15. Iumillo × Hope	11	5	15	11	2	0	1	0	0	0	0	0
<i>T. vulgare</i> × <i>Timopheevi</i>												
16. Canus × Timopheevi	18	5	13	4	5	0	2	0	1	0	1	0

* Compare these with mean numbers given in Table II.

TABLE IV

FREQUENCIES (%) OF PMC WITH SELECTED* CHROMOSOME ARRANGEMENTS IN F_1
T. vulgare × *T. dicoccum*

Chromosome arrangement					McMurachy × Khapli	Marquis × Khapli	Hope × Khapli	McMurachy × Vernal	Marquis × Vernal	Hope × Vernal
V	IV	III	II	I						
			15	5	0.5	—	0.2	0.4	—	—
			14	7	35.5	47.2	53.6	42.4	65.8	66.2
		1	13	6	10.5	5.2	11.1	6.5	3.3	1.8
	1	—	12	7	10.5	2.1	4.8	6.5	0.5	0.2
		2	12	5	—	0.3	0.4	—	—	—
	1	1	11	6	0.5	—	—	0.9	—	—
1	—	—	11	8	0.5	—	—	—	—	—
Number of PMC analysed					200	326	541	231	480	544
PMC with one or more multiple associations, %					34.5	13.5	20.9	23.4	5.2	2.9
Number of different pairing arrangements observed					16	11	14	15	10	9

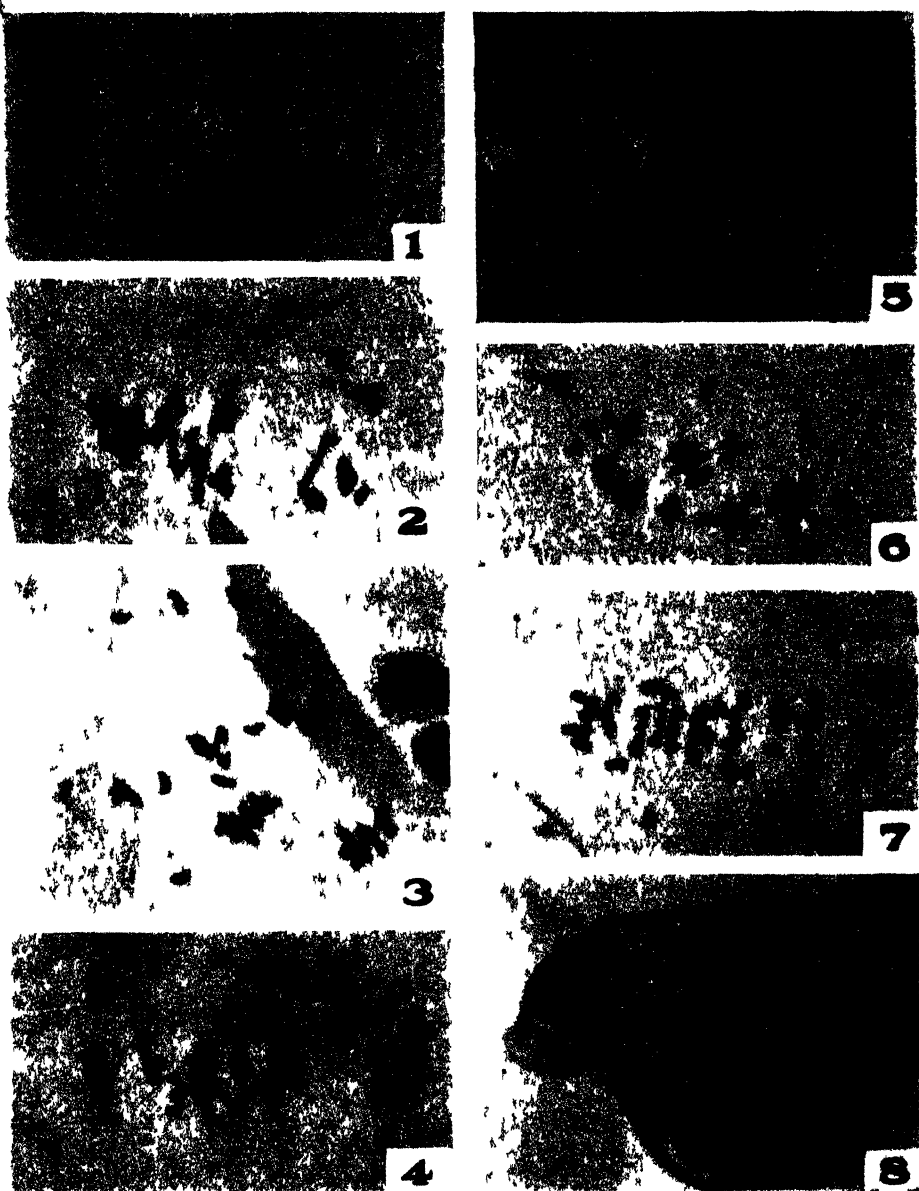
* Explanation in the text.

chromosome from the first (or second) set and the mate of the latter forming an association of three with a pair of the second (or first) set. It would still mean that the extra chromosome is associated with the others, which is the main issue.

Many pollen mother cells contain one (or more) associations of three chromosomes but have seven or more univalents plus the requisite number of pairs or multiple associations. In such cases it is not known whether the associations of three involve one (or more) of the extra chromosomes—they may include only chromosomes derived from the “primary sets”. It is obvious that the possibility of multiple associations (or even of pairs) through autosyndesis cannot be entirely ruled out. In the hybrid involving *T. Timopheevi* some are certainly due to autosyndesis since multiple associations are found in 10% of the pollen mother cells examined in this parent.

All associations of three, five, and six chromosomes were chains. The associations of four chromosomes were chains in the hybrids Iumillo × Hope and Marquis × Iumillo, and the hybrids involving Khapli and Vernal. Rings as well as chains of four chromosomes were found in all plants of the three hybrids involving Pentad (Fig. 2). The frequencies of rings of four in these hybrids were as follows:

Hybrid	Number of associations of four chromosomes	Percentage of rings of four
McMurachy × Pentad	200	11.5
R.L. 1544 × Pentad	369	27.6
Marquis × Pentad	803	38.1



Photomicrographs of pollen mother cells in F_1 pentaploid wheat hybrids. Aceto carmine preparations $\times 560$. FIG 1 McMURACHY \times PENTAD — one association of three 13 pairs and six univalents. FIG 2 MARQUIS \times PENTAD — three associations of four chromosomes (one ring), one association of three chromosomes, seven pairs, and six univalents. FIG 3 McMURACHY \times PENTAD — one association of five chromosomes, 10 pairs, and 10 univalents. FIG 4 MARQUIS \times PENTAD — one association of six chromosomes, one association of four chromosomes, nine pairs, and seven univalents. FIG 5 McMURACHY \times IUMILLO. Note the univalent in the middle of the cell broken at or in the spindle fibre attachment region. The two arms of the fragmented chromosome are being drawn to opposite poles. FIG 6 IUMILLO \times RL 729 — 14 pairs and seven univalents. FIG 7 HOPE \times VERNAL — 13 pairs and nine univalents. FIG 8 MARQUIS \times PENTAD — second division inversion bridge and accompanying fragment.

TABLE V
FREQUENCIES (%) OF PMC WITH SELECTED* CHROMOSOME ARRANGEMENTS IN F_1 *T. vulgare* \times *T. durum*

Chromosome arrangement					Marquis \times Pentad	McMurachy \times Pentad	R.L. 1544 \times Pentad	McMurachy \times Iumillo	Iumillo \times R.L. 729	Marquis \times Iumillo	Iumillo \times Hope	R.L. 1544 \times Iumillo	Marquillo \times Iumillo
VI	V	IV	III	II	I								
					15	5	—	—	1.2	—	0.3	—	—
					14	7	4.5	51.4	60.0	64.2	67.5	73.7	91.3
					13	6	1.4	5.4	13.3	12.5	8.5	6.3	2.5
					12	5	0.3	—	—	0.2	0.3	0.3	—
					12	7	25.1	37.1	—	0.2	0.8	—	—
					11	6	6.1	13.7	—	0.2	0.3	—	—
					11	6	0.9	1.0	—	—	0.3	—	—
					10	5	0.3	—	—	—	—	—	—
					10	5	0.4	0.6	—	—	—	—	—
					10	7	20.4	0.3	—	—	—	—	—
					9	4	0.1	—	—	—	—	—	—
					9	6	0.1	—	—	—	—	—	—
					9	6	1.6	—	—	—	—	—	—
					8	7	0.4	—	—	—	—	—	—
					8	6	0.6	—	—	—	—	—	—
					8	8	0.1	—	—	—	—	—	—
					8	8	0.1	—	—	—	—	—	—
					9	7	0.9	—	—	—	—	—	—
					9	7	—	—	—	—	—	—	—
Number of PMC analysed					706	313	542	259	165	561	400	395	402
PMC with one or more					92.6	75.1	83.8	13.1	16.9	16.4	10.0	8.1	2.7
multiple associations, %					—	—	—	—	—	—	—	—	—
Number of different pairing					49	22	24	10	6	10	10	9	6
arrangements observed					—	—	—	—	—	—	—	—	—

* Explanation in the text.

A chain of five chromosomes was seen in two pollen mother cells of the hybrid Canus \times *T. Timopheevi*, in three of the hybrid McMurachy \times Pentad (Fig. 3), and in 16 of the hybrid Marquis \times Pentad. A chain of six chromosomes was seen in one pollen mother cell of the hybrid Canus \times *T. Timopheevi*, in two of the hybrid McMurachy \times Pentad, and in 10 of the hybrid Marquis \times Pentad (Fig. 4).

(ii) *Inversions*

Material showing first anaphase figures was available for 28 plants representing 13 of the 16 hybrids. In every plant an inversion bridge and an accompanying fragment were detected in from one to five pollen mother cells. In addition, two bridges and two fragments were seen in one or more pollen mother cells of the hybrids: Iumillo \times Hope, Marquis \times Iumillo, McMurachy \times Iumillo, and McMurachy \times Pentad. Further details on inversions are given in the section on "Tetrad Studies".

(iii) *Chromosome Breaks*

Unpaired chromosomes broken at the spindle fibre attachment region were found at the first meiotic division in all hybrids except those of Iumillo \times Hope. Altogether, 39 such broken chromosomes were detected. In two chromosomes, both fragmented arms bore the spindle fibre attachment region (Fig. 5). In all others, only one arm bore the attachment region. Two such broken univalents were found in one pollen mother cell of several hybrids and four occurred in one pollen mother cell of a Hope \times Vernal hybrid.

Because of the appreciable number of unpaired chromosomes that were broken at or in the attachment region during first anaphase, it was decided to examine carefully the behaviour of the univalents before they became orientated on the equatorial plate. In these hybrids the univalents lag behind the paired chromosomes (Fig. 6). A split is evident in the unpaired chromosomes at the same time that it appears in members of the bivalents or multiple associations (Fig. 7).

(iv) *Aberrant Pollen Mother Cells*

Pollen mother cells with more or less than 35 chromosomes were seen in some hybrids. Nuclei with 70 chromosomes were seen five times—in two plants each of F_1 Hope \times Vernal and Hope \times Khapli, and in one plant of F_1 R.L. 1544 \times Iumillo. In one the chromosomes were arranged as 27 bivalents and 16 univalents. In one plant of F_1 Marquis \times Vernal, two pollen mother cells contained only 28 chromosomes, arranged as 6 bivalents and 16 univalents.

(b) *Tetrad Studies*

The observations on material showing the second meiotic division were confined for the most part to late anaphase, early telophase, or young pollen tetrad stages. The latter stage is satisfactory for counts of micronuclei and for the observation of inversion bridges. The results of these studies are summarized in Table VI, which includes fertility data kindly supplied by Dr. R. F. Peterson.

TABLE VI

IRREGULARITIES DETECTED IN YOUNG POLLEN TETRADS, AND FERTILITY, OF F_1 PENTAPLOIDS

Hybrid	Number of plants	Tetrads with micronuclei, %	Mean number of micronuclei per tetrad*	Tetrads with inversion bridges, %	Fertile florets, %
<i>T. vulgare</i> × <i>dicoccum</i>					
1. Hope × Vernal	4	92.7	2.2	0.5	52.5
2. Marquis × Vernal	4	93.7	2.5	1.1	46.4
3. McMurachy × Vernal	3	84.0	1.8	0.7	7.0
4. Hope × Khapli	5	99.6	5.0	1.0	0.8
5. Marquis × Khapli	3	99.3	3.1	3.7	0.0
6. McMurachy × Khapli	3	97.7	3.5	0.5	0.0
<i>T. vulgare</i> × <i>durum</i>					
7. R.L. 1544 × Iumillo	6	85.7	1.6	7.4	68.9
8. Marquis × Iumillo	2	88.0	1.9	1.5	73.6
9. McMurachy × Iumillo	3	79.0	1.6	5.2	45.2
10. R.L. 1544 × Pentad	5	82.0	1.7	6.9	11.8
11. Marquis × Pentad	4	92.7	2.1	6.5	24.6
12. McMurachy × Pentad	1	100.0	3.2	5.5	27.8
13. Marquillo × Iumillo	5	97.1	3.0	5.8	?
14. Iumillo × R.L. 729	3	93.5	2.3	3.1	?
15. Iumillo × Hope	3	98.7	3.0	1.3	?

* Necessary difference between means for significance at the 5% point is 0.6.

(i) *Micronuclei*

Variation in the numbers of micronuclei (per 200 tetrads) in 54 plants of 15 hybrids was analysed statistically using the "Analysis of Variance" method; the F value was found to be 41.71. According to Snedecor (26) an F value of 2.66 is required for significance at the 1% point. A difference of 0.59 between the means for the hybrids is significant at the 5% point (Table VI).

(ii) *Inversions*

Inversion bridges were found in one or more pollen mother cells of all plants studied (Fig. 8). Since it was not possible in many instances to determine the source of all micronuclei in a tetrad (some may be due to the rounding up of chromosome fragments, of whole chromosomes, or of inversion fragments) no record of the latter is given.

The frequencies of tetrads in which inversion bridges occurred are recorded in Table VI. It is seen that the frequency of pollen mother cells with one or more bridges varied greatly in the different hybrids—from a low of 0.5% in F_1 Hope × Vernal and McMurachy × Khapli to a high of 7.4% in F_1 R.L. 1544 × Iumillo. One and two bridges were detected in pollen mother cells of all hybrids involving the varieties of *T. dicoccum*. One, two, and three bridges were seen in some pollen mother cells of the following hybrids: Marquis

× Khapli, Marquillo × Iumillo, McMurachy × Iumillo, R.L. 1544 × Pentad, and Marquis × Pentad. As many as four bridges were seen in several pollen mother cells of F_1 R.L. 1544 × Iumillo.

(c) *Fertility*

It is seen from the data in the last column of Table VI that the hybrids 1 to 12, inclusive, fall into four fertility groups, depending mainly on the particular 28-chromosome variety involved, although it is evident that within each group there are differences attributable to the various 42-chromosome varieties concerned. According to the data obtained, the hybrids rank as follows:

Hybrids involving:	Fertility, %	Fertility rank
<i>T. durum</i> var. Iumillo	45.2 – 73.6	1 (most fertile)
<i>T. dicoccum</i> var. Vernal	7.0 – 52.5	2
<i>T. durum</i> var. Pentad	11.8 – 27.8	3
<i>T. dicoccum</i> var. Khapli	0.0 – 0.8	4

One exception is F_1 McMurachy × Vernal with a very low percentage of fertile florets. It is noteworthy that the two varieties in each of the 28-chromosome species fall into distinctly different fertility grades. The hybrids involving Khapli are least fertile and have the largest mean numbers of micronuclei per tetrad. The reverse is true for the hybrids involving Iumillo, but, in general, it cannot be stated that the percentage of fertile florets varies inversely as the number of micronuclei (Table VI).

Comparison of Pairing Behaviour in the Hybrids

As far as the writer is aware, this is the first study of its kind to include check crosses as standards for comparison. *T. vulgare* var. R.L. 1544 is *genetically* related to *T. durum* var. Iumillo since it is a selection from the cross Marquis × Iumillo. It must have, therefore, whole chromosomes or segments of chromosomes derived from the 28-chromosome parent. The data obtained from the cross R.L. 1544 × *T. durum* var. Iumillo, should assist in establishing criteria that will indicate relationships between the other 42- and 28-chromosome wheats. *T. vulgare* var. Hope is *genetically* related to *T. dicoccum* var. Vernal, so the cross Hope × Vernal should provide data similarly useful. On the other hand, *T. vulgare* var. Marquis is known to be not *genetically* related to either *T. dicoccum* or *T. durum*. Thus, the crosses of Marquis with varieties of these species should provide data complementary to those involving R.L. 1544 and Hope.

The relationship of one wheat to another might result in one of two main sets of conditions in the chromosomal constitution. Assuming that variety x has received chromatin from variety y and is therefore related to y , x might have

- (1) whole y chromosomes or else blocks of y genes in their normal position on the corresponding chromosomes; or
- (2) translocated blocks of y genes.

In either case variety x must now be considered more closely related to variety y than it was originally. The first condition would tend to increase the number of pollen mother cells having 14 bivalents and 7 univalents in the F_1 of variety $x \times$ variety y , whereas the second condition would have the reverse effect. Similarly, the first condition would tend to reduce, whereas the second would tend to increase, the number of multiple associations of chromosomes in the F_1 . This raises the question of which of the two opposite criteria of relationship is valid in this material. This can be tested by comparing chromosome behaviour in hybrids between parents of known genetic relationship. Fertility of the F_1 may also be an important criterion.

Hybrid	PMC with $14_{II} + 7_I$, %	Average number per PMC of chromosomes involved in multiple associations	Fertile florets, %
<i>T. vulgare</i> \times <i>T. durum</i>			
R.L. 1544 \times Iumillo	73.7	0.26	68.9
R.L. 1544 \times Pentad	12.4	3.59	11.8
<i>T. vulgare</i> \times <i>T. dicoccum</i>			
Hope \times Vernal	66.2	0.09	52.5
Hope \times Khapli	53.6	0.69	0.8

The hybrid R.L. 1544 \times Iumillo has a much greater frequency of pollen mother cells with 14 bivalents and 7 univalents and a much smaller average number of chromosomes involved in multiple associations than has the hybrid R.L. 1544 \times Pentad. The hybrid Hope \times Vernal has a greater frequency of pollen mother cells with 14 bivalents and 7 univalents and a significantly lower average number of chromosomes involved in multiple associations than has the hybrid Hope \times Khapli. It seems, therefore, that the first condition mentioned above outweighs the second in importance as establishing criteria of relationship between the wheats discussed in this study. It is also seen that the F_1 between *genetically* related varieties is much more fertile than is the F_1 between unrelated varieties.

The above comparisons indicate that in this material the more closely the 42- and 28-chromosome wheats are related, the greater will be the expected frequency of pollen mother cells with 14 pairs and 7 univalents and the smaller the frequency of multiple associations. Thus, the comparisons as given above for hybrids involving R.L. 1544 (*genetically* related to *T. durum* var. Iumillo) or Hope (*genetically* related to *T. dicoccum* var. Vernal) should obtain if there is no genic sterility as defined by Dobzhansky (3, p. 263). Of course, auto-syndesis may mask the picture and its possibility (as discussed previously) must be kept in mind.

On the basis of the results obtained from a comparison of chromosome behaviour and fertility in the hybrids involving R.L. 1544 and Hope, an attempt can be made to establish the source of rust resistance of McMurachy's Selection.

Hybrid	PMC with $14_{II} + 7_I$, %	Average number per PMC of chromosomes involved in multiple associations	Fertile florets, %
<i>T. vulgare</i> × <i>T. durum</i>			
McMurachy × Iumillo	51.4	0.41	45.2
McMurachy × Pentad	11.2	3.65	27.8
<i>T. vulgare</i> × <i>T. dicoccum</i>			
McMurachy × Vernal	42.4	0.82	7.0
McMurachy × Khapli	35.5	1.25	0.0

Of the hybrids studied, McMurachy's Selection × Iumillo has the greatest frequency of pollen mother cells with 14 pairs and 7 univalents, the lowest average number of chromosomes involved in multiple associations, and it is the most fertile. The three factors may be taken to indicate that McMurachy's Selection is more closely related to *T. durum* var. Iumillo than to the other 28-chromosome wheats included in this study.

T. vulgare var. Marquis is a much older 42-chromosome wheat than either R.L. 1544 or Hope, but it is interesting to attempt to set up a relationship between Marquis and the 28-chromosome wheats studied.

Hybrid	PMC with $14_{II} + 7_I$, %	Average number per PMC of chromosomes involved in multiple associations	Fertile florets, %
<i>T. vulgare</i> × <i>T. durum</i>			
Marquis × Iumillo	64.2	0.51	73.6
Marquis × Pentad	4.5	5.85	24.6
<i>T. vulgare</i> × <i>T. dicoccum</i>			
Marquis × Vernal	65.8	0.16	46.4
Marquis × Khapli	47.2	0.44	0.0

The gene arrangement in chromosomes of Marquis appears to resemble that in Vernal more nearly than that in the other three varieties. This probably accounts for "the greater ease with which *vulgare* characters can be transferred to 14-chromosome plants in *vulgare-dicoccum* [i.e., Vernal] than in *vulgare-durum* [i.e., Iumillo] crosses" as shown by Thompson *et al.* (28). But, fertility is greatest in the Marquis × Iumillo hybrids. This raises the question as to which is the more important criterion in establishing relationships between species—similarity of gene arrangement or fertility in the F_1 hybrid. The former is probably the more significant since fertility is so often affected by small differences.

The variety Pentad deserves comment. It was introduced from Russia in 1903 by Prof. H. L. Bolley of the North Dakota Agricultural Experiment Station. According to Clark and Bayles (2), "Pentad is distinct from all other commercial varieties of durum wheat grown in the United States because of its rust resistance, white glumes, and red kernels. The kernels are smaller, squarer at the brush end, and more pointed at the germ end than kernels of the other durum varieties Its quality has been found inferior, however, to that of other durum varieties." Iumillo is not one of the "other commercial varieties" referred to by Clark and Bayles, but there is no doubt that Iumillo and Pentad differ markedly in the constitution of their chromosomes as evidenced by the pairing behaviour in hybrids with the three 42-chromosome wheats R.L. 1544, Marquis, and McMurachy (Tables II, V, and VI). Fertility of the F_1 hybrids involving Pentad is significantly lower than that of hybrids involving Iumillo (Table VI, last column).

Discussion

The results of the present study are in sharp disagreement with the earlier work on chromosome behaviour in pentaploid wheat hybrids. A detailed review of the previous results is unnecessary since the whole situation as it appeared in 1935 has been thoroughly treated by Aase (1) and Hector (6). Discussions of the "genom analysis" of Kihara and his school have been made by Dobzhansky (3) and Sansome and Philp (24).

In the review by Aase (1) there is no reference to multiple associations in hybrids between wheats with different chromosome numbers. She concluded: "The number of pairs at meiosis approaches that of the parents with the lowest chromosome number. The number of probable pairs is most complete, and ring pairs predominate in the 21×14 -chromosome hybrid." In so far as the number of probable pairs is concerned, the present results are in agreement with this statement. But it does not tell the whole story.

Hector (6, p. 163) wrote: "At the reduction division, the behaviour of the chromosomes is almost uniform. The 35 chromosomes form 14 bivalents and 7 univalents; Sax [25] reported a case of nine univalents and Kihara and Nishiyama [13] the rare occurrence of trivalents." In the present material, the behaviour of the chromosomes is not uniform (Tables III, IV, and V). In the Marquis \times Iumillo hybrids, 90% of the pollen mother cells contained 14 bivalents and 7 univalents whereas that pairing arrangement occurred in less than 5% of the pollen mother cells of plants of Marquis \times Pentad. In the latter, 25% of the pollen mother cells contained 1 association of four chromosomes, 12 bivalents, and 7 univalents and 20% contained 2 associations of four, 10 bivalents, and 7 univalents. Six different pairing arrangements of the 35 chromosomes were observed in Marquillo \times Iumillo hybrids and 49 in Marquis \times Pentad.

On the basis of pairing relationships published in the past, the following hypothesis has been suggested (4): the Einkorn ($2n = 14$), Emmer ($2n = 28$), and Vulgare ($2n = 42$) groups have respectively, one, two, and three sets

of seven chromosomes that differ from each other. The sets have been denoted *A*, *B*, and *C* by some workers (27) and *A*, *B*, and *D* by others (cf. 3). Thus, Einkorns are represented as *AA*, Emmers as *AABB*, and Vulgares as *AABBCC* or *AABBDD*. But, as early as 1929, Jenkins (11) concluded that the situation is much more complex. In 1931, Thompson (27) reviewed the work on the various groups (including *Aegilops* species) that had been published at that time. His conclusion was: "In view of all the difficulties, disagreements, inconsistencies, and possible sources of error much more work must be done before a satisfactory scheme of chromosome homologies, embracing all the species and reflecting botanical and genetical relationships, can be drawn up."

Nevertheless, apart from the rare occurrence of trivalents referred to above (13), it was not until *T. Timopheevi* ($2n = 28$) was crossed with other wheats and the pairing behaviour of chromosomes in the hybrids was found to be very complex (12, 14) that real difficulty appeared in the minds of the workers who attempted to maintain the simple scheme suggested by the above hypothesis. The difference between *T. Timopheevi* and the other Emmers was thought to be in the "*B* set" and Kihara and Lilienfeld (12) suggested that the "formula" of this species is *AG*. Kostoff (14), however, decided that since there was some "affinity" between the *B* and *G* sets the formula should be *Aβ*. The occurrence of multiple associations in *T. Timopheevi* itself complicates the situation, and it is very likely that chromosomes in both the "*A*" and "*G*" (or "*β*") sets have become differentiated phylogenetically. At least some of the multiple associations observed in hybrids involving this species must be autsyndetic. This is probably true for other pentaploid hybrids as well.

The present data are not sufficiently extensive to assign such symbols to the various chromosome sets, and, in fact, the results indicate that such a procedure cannot possibly be more than an approximate index of the actual pairing behaviour observed. The writer's data (Tables II and III) show definitely that *T. Timopheevi* is not an exception to any general rule, but that it differs from the other 28-chromosome wheats in degree only.

Dobzhansky (3), while admitting that the hypothesis has on the whole been satisfactory, concluded that "its schematic simplicity is largely a thing of the past" and stated that "the occurrence of allopolyploids implies as an antecedent a differentiation of the chromosomes in the ancestral species by translocation, inversion, and other means" (p. 219). According to Sansome and Philp (24), the rare transference of certain characters may be explained by assuming that one of the "extra" seven chromosomes of Vulgare occasionally pairs with an Emmer chromosome. This conclusion was recently reached on genetic grounds by Peterson and Love (21). There is ample evidence that this does occur in all the hybrids examined in this study.

The writer's results indicate that all the wheat varieties used, whether of the 28- or 42-chromosome group, differ to a greater or lesser degree in the arrangement of chromosome segments. This is evidenced in the hybrids

the presence of varying numbers of multiple associations (associations between chromosomes of the so-called "primary *A* and *B*" sets, and associations between chromosomes of the "primary" and "secondary" sets) and inversions in all the hybrids examined. This is not in agreement with the conclusion reached by Peto (22) from his cytological study of *Agropyron-Triticum* hybrids. The present material, with a smaller number of chromosomes, is relatively easier to analyse, and the large numbers of complete nuclei recorded in each hybrid allow no doubt as to the validity of the conclusion given above. On the other hand, the difficulty encountered in this study in attempting to establish criteria indicating relationships between certain of the 28- and 42-chromosome wheats by means of pairing behaviour and fertility in the hybrids (even where the *genetic* relationship is known) indicates that the utmost caution must be used in drawing phylogenetic conclusions on the basis of such data.

Several questions are posed by the kind and frequency of multiple associations found in the various pentaploid hybrids. The frequency of pollen mother cells with multiple associations varies in the different hybrids from less than 3% in Hope \times Vernal and Marquillo \times Iumillo to more than 90% in Marquis \times Pentad (Tables IV and V). If three chromosomes in the different sets can (and do with varying frequencies) unite to form a multiple association why do they not always, or nearly always, do so in any one cross? This situation is very different from that found in artificially induced translocations. Why do the multiple associations in this hybrid material so often form chains instead of rings? And finally, why are associations of three chromosomes so much more abundant than those of four? The most probable explanation seems to be that many of the multiple associations arise through pairing of chromosomes that are phylogenetically similar but not strictly homologous. Huskins (9) has referred to such chromosomes as "*homælogous*, signifying similarity but not identity." Such pairing would not be nearly as precise as that due to homology in the strict sense, and so the frequency of multiple associations (and indeed even of pairs) involving certain chromosomes would be variable in the interspecific hybrids. The genetic results obtained by Thompson *et al.* (28) showed that genes with similar effects on many characters were present in all the chromosome sets of *T. vulgare*. The fact that chains of three chromosomes are more abundant than chains of four in the pentaploid hybrids supports their conclusions based on genetic data.

Observations of the behaviour of the unpaired chromosomes at meiosis in 13 of the pentaploid hybrids show that breaks occur at or in the spindle attachment region. As a result of these breaks one or both arms of the fragmented chromosome may bear a spindle fibre attachment region (Fig. 5) and thus may be able to participate in succeeding nuclear divisions. From a study of the behaviour of such telocentric chromosomes in maize, Rhoades (23) concluded, "The production of secondary or isochromosomes at meiosis from the telocentric chromosome and its loss and modification in somatic tissue show that a terminal centromere is unstable." Love (18) has reported

similar evidence (at meiosis) in derivatives of pentaploid wheat hybrids. But it must be emphasized that the attachment region of unpaired chromosomes is also quite unstable, at least during meiosis. This has been shown in the present study and elsewhere by the writer (16, 17, 18, and unpublished data) and others (cf. Rhoades (23)). A pair of homologous telocentric chromosomes, on the other hand, appeared to be as stable as the normal chromosomes in the complement of a winter wheat variety under investigation for five generations (Love (15), and unpublished data). Rhoades (23) reported that "the telocentric chromosome [in maize] underwent normal meiotic behaviour when it was a member of a trivalent group." Abnormal behaviour of telocentric chromosomes during meiosis alone cannot explain their absence from the normal complement of plants and animals, and the investigation of Rhoades when considered in relation to the observations of the writer strongly suggests that it is in somatic divisions that the telocentric chromosome is at a disadvantage.

Acknowledgments

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DEVELOPMENTAL STUDIES OF THE APPLE FRUIT IN THE VARIETIES MCINTOSH RED AND WAGENER

II. AN ANALYSIS OF DEVELOPMENT¹

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Abstract

Growth in the various tissues of the fruit of a McIntosh Red and a Wagener tree, both self-pollinated, is compared. For several days succeeding pollination no increase in fruit size is apparent. Fertilization is followed by general cell division and cell enlargement. The period of cell division varies with the tissue and with the variety. Final cell size is reached first by the cells of those tissues near the centre of the apple. Impressed upon the fundamental pattern of growth is the localized activity of the primary vascular bundles, the cambia of which add cells to the ground tissue. Angulation in the Wagener is accentuated by this activity. With the exception of cells of the epidermis, final cell size is approximately equal in comparable regions of the two varieties. Differences in regional extent are due to differences in numbers of cells in that region.

Specific size and form of an organ are achieved by the interplay of those integrating and co-ordinating processes which control development. Since such processes are dynamic, successive stages of development should form the basis of an analysis of growth in that organ. An initial stage in the study of the attainment of specific size and form in the fruit of the apple varieties, McIntosh Red and Wagener, was presented in an investigation (4) of the development of the vascular anatomy in these varieties. A logical sequence to this study is the investigation of the growth of the fruit as a whole and of the various tissues comprising that fruit. With this end in view, there were assembled measuremental data on the direction and extent of localized growth (involving cell division and cell enlargement), the differentiation and organization of the cells into tissues, and the relative importance of these tissues in the production of the mature fruit. The consideration and the interpretation of these data are reported in this paper.

As far as the authors are aware, previous investigations of the relation between cell size and organ size in apples have been made on the mature fruit alone. In the variety Bramley's Seedling, Smith (6) found a centrifugal "gradient" of differences in the sizes of the cells of the various regions from pith to cortex. In a later paper (7) he attributed final fruit size to (a) the amount of cell multiplication, and (b) the degree of cell enlargement, wherein

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either factor might be the dominant one. Houghtaling (2) in a study of the developmental anatomy of tomatoes, showed that growth in the early stages is due chiefly to an increase in cell number, later growth to an increase in cell size, and that both processes operate more extensively to produce the larger-sized fruits. In an analysis of the genetical basis of size inheritance in the tomato, MacArthur and Butler (3) state that the size differences of different species or varieties are due to rate genes that control cell number and cell size. Differences in ovary size at anthesis are due to differences in cell number, whereas differences in the size of the more mature fruit are due also to the fact that maximum cell expansion varies greatly in the several varieties during postanthesis. Sinnott's investigations on the morphogenesis of *Cucurbita Pepo* (5) take into consideration the various tissues comprising the fruit. In 12 races of squash, larger fruit size at maturity was due to a greater duration of cell division and to a greater amount of cell expansion. Various tissues increased at different rates.

Materials and Methods

At the Experimental Station, Kentville, N.S., collections of developing fruits were made throughout the growing seasons of 1935 and 1936 from a selfed McIntosh Red and a selfed Wagener tree pollinated in 1935 on June 8 and in 1936 on May 26. Fixation and staining methods were those used by

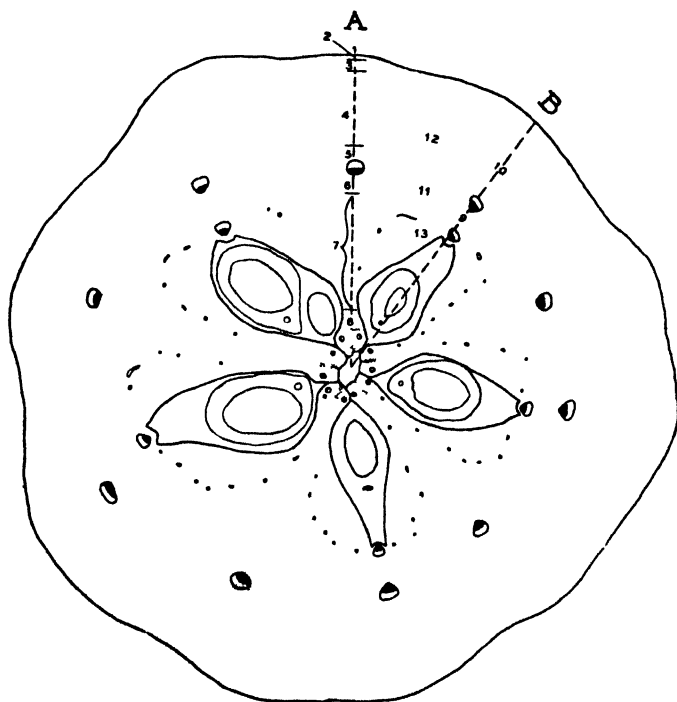


FIG. 1. Diagram of median transverse section of the apple showing petal and sepal radii A and B, and the location of the Regions 1 to 13 used in the measurements.

MacArthur and Wetmore (4). Only the median transverse sections were used in the measurements. An Edinger drawing apparatus was used to project the sections on paper. Cell size of tissues along and between the petal and sepal bundle radii (*A* and *B*, Fig. 1) were measured. Calibrated rules had been prepared for each combination of ocular and objective. Owing to the fact that there was considerable variability in the diameters of the cells in any one tissue, due principally to the differences of levels at which the cells were cut, the very small cells were avoided. Sample lots of 10 cells were selected in each region. Each of these cells was measured on its longest diameter, and again at right angles to this, and the two dimensions were averaged. Mean diameters of the cells of each region studied, radial widths of those regions, and the radial dimension of each entire section were the measurements used in the computations.

Observations

TISSUE CHARACTERS

Region 1

At no time in the stages examined do the epidermal cells (Region 1) of the McIntosh reach the size of those of the Wagener. The radial diameter of the epidermal cells of the McIntosh remains approximately the same from one week previous to anthesis until six days after pollination when an increase is then initiated. Three days after pollination the epidermal cells of the Wagener initiate their slow, steady increase in radial diameter. The tangential diameters in both varieties are equal and remain about the same until the end of June. From June 24 on, this latter diameter in both varieties increases and finally surpasses the radial diameter. The present observations confirm those of Tetley (8), that throughout the period of growth the layer of cutin limiting the external face of the epidermal cells gradually thickens.

Region 2

The subepidermal layer (Region 2) is at first one or two cells in thickness. In the very early stages there is no definite orientation of the longest diameter of these cells. At the time of increase in the radial thickness of the epidermis (see above), the subepidermal cells are oriented so that their longest diameter is perpendicular to the radius of the apple. Shortly after anthesis, the radial extent of this tissue increases slightly, first by periclinal cell division, then by cell enlargement. Cell enlargement is mainly effected by the tangential elongation of the cells since the radial diameters of these cells remain practically the same. The cell walls become very thick and no intercellular spaces are visible until early in August.

Region 3

In the outer cortex (Region 3) the cells are more or less isodiametric at the time of pollination. The outer boundary is recognizable early* by the large intercellular spaces similar to those reported by Bonne (1) for *Pyrus*

* The earliest buds studied were collected April 29. Already, the intercellular system was apparent in this region.

communis and termed "la zone corticale lacuneuse." In the bud and young fruit this layer with its extended intercellular areas resembles the mesophyll tissue of a leaf. As the cells divide and enlarge this distinction is lost. The outer cortex is interpenetrated by numerous small vascular bundles each of which is surrounded by a sheath of parenchyma. The cells of this tissue merge into those of the cortex.

Region 4

The cortex proper (Region 4) is traversed by the larger branches of the anastomosing vascular system. Early in ontogeny the cells are nearly isodiametric. Later, they become roughly elliptical in shape and a radial orientation is visible. The arrangement of the cells in this tissue and the orientation of the cells themselves are probably due to the centrifugal direction of growth. This region is one showing great increase in absolute extent during the growth of the fruit.

Region 5

In the bud there is no definite orientation of this tissue which limits the outer face of the petal bundle. The cambium of the bundle, which is active in the later stages of growth, cuts off parenchyma cells externally. With this activity radial orientation is initiated.

Region 6

This is the region that limits the inner face of the petal bundle. The activity of the cambium of the bundle in cutting off parenchyma cells internally makes this region the mirror image of Region 5.

Region 7

The cells of the ventral lobe of the carpel region are radially oriented from the time of inception of the ventral lobe (4). In the later stages of growth, the staining reaction of this region differs from that of the outer tissues; starch grains are not as numerous as in Region 4, and, after the cells have reached their maximum enlargement, further increase in the radial dimension of the tissue is due to the enlargement of the intercellular spaces. This increase causes distortion of the radial arrangement of the cells.

Region 8

This tissue is made up of small cells, diversely oriented. Its distance from the centre of the apple varies according to the length of the indentations of the styler canal. These indentations in Wagener are indicated either by an opening into the ventral lobe or by tangentially oriented, interlaced cells which in the later stages of development generally change to stone cells. In the McIntosh, because of: (a) the irregular orientation of the ovules (4), and (b) the frequent occurrence of one concentric ventral bundle serving two adjacent locules and occupying a median position in the ventral lobe, the indentations of the styler canal in the carpel region are often absent.

Region 9

The tissue of Region 9, on Radius *B*, lies between the dorsal carpel bundle and the sepal bundle. The cells are oriented in a manner similar to that in Region 6. Its increase in extent is due to the activity of the cambium of these two bundles in cutting off parenchyma cells.

Region 10

The cells of the cortical region on the sepal bundle radius are in all respects similar to those of Region 4 on the petal radius.

Region 11

This region differs in the two varieties. It presents an angular outline, extending into the cortical tissue between sepal and petal bundle in the McIntosh. In the Wagener it is roundly lobed. The cells have no definite orientation in the early stages, but in the later stages show a certain tangential orientation, apparently brought about by the increase of pressure during the active growth of the cortex externally, and the ventral carpel lobes internally. The cells of Region 11 stain heavily.

Region 12

The tissue of Region 12 is similar to and continuous with that of Regions 4 and 10.

Region 13

This is the fleshy region of the carpels, extending from the endocarp to the carpellary network. It has a greater extent in the Wagener than in the McIntosh. Those cells that lie close to the endocarp are at first cubical. During enlargement they become several times longer than wide and the longer diameter is roughly parallel to the cartilaginous endocarp. The outer cells of the tissue divide periclinally and are finally indistinguishable from the cells of Region 7.

Interpretation of Results

A graphical summary of enlargement along Radius *A* is given in Fig. 2, where radial increase is plotted against time. At the time of anthesis the Wagener fruit is slightly larger than that of the McIntosh. In the former, on the third day after pollination, a slow increase in size begins. For the succeeding three days the increase is gradual, but with the drop of the petals the increase becomes rapid and continues at approximately this rate until towards the completion of growth.

On the other hand, the McIntosh shows no corresponding increase in size in the early stage after pollination, but in the succeeding stage a similar abrupt change is suggested though the material was not sufficient to demonstrate this point. The delayed growth response in the McIntosh may be associated with its known high self-incompatibility.

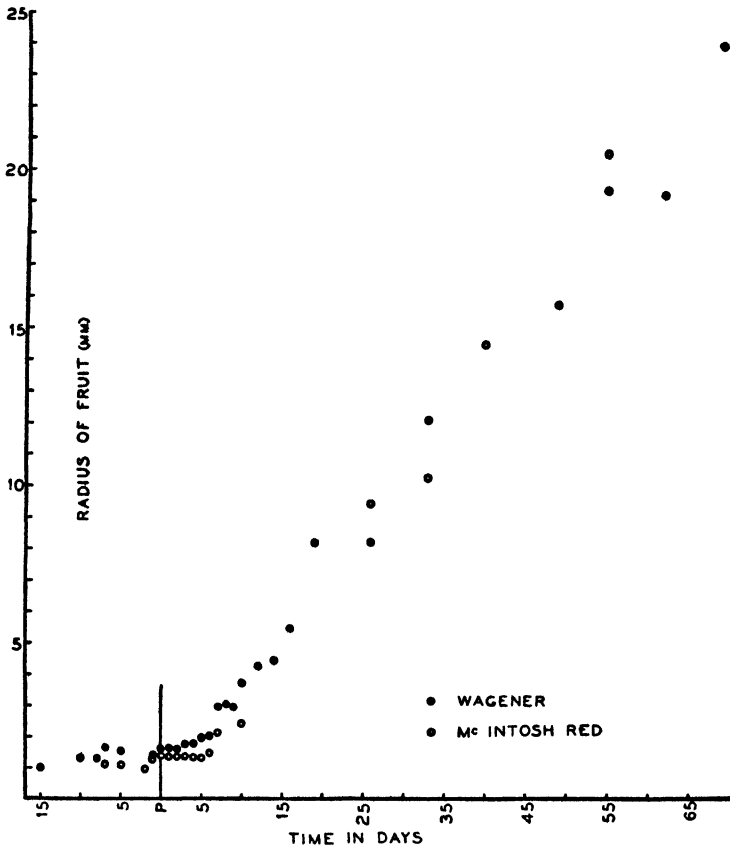


FIG 2. Increase of radial extent of the fruits of McIntosh Red and Wagener with respect to time. *P* = pollination

Six days after pollination the unfertilized ovules begin disintegration. Later collections of fruits with no fertilized ovules show progressive stages of fruit disintegration, this disintegration beginning in the outer cortex. These are the fruits that constitute the first "drop". In contrast, in those fruits with fertilized ovules the sudden increase of growth is apparent after the slow period. The rate of growth with respect to time is approximately the same in the two varieties. The tissues that show the greatest increase in size in the early stages are Regions 4 and 7. Later, the cambium of the petal bundle adds to the ground tissue by increasing Regions 5 and 6. Since the line of division between the cortex and the carpel region follows the inner limits of Region 6, Regions 1 to 6 inclusive have been grouped additively for the purpose of comparison with Region 7. The total width of: (a) these outer tissues (Regions 1 to 6 inclusive), and (b) Region 7 are independently plotted against time for the McIntosh (Fig. 3) and for the Wagener (Fig. 4). No attempt has been made to fit a curve to the points. Variation from a smooth curve of growth is to be expected since (a) these are the results of

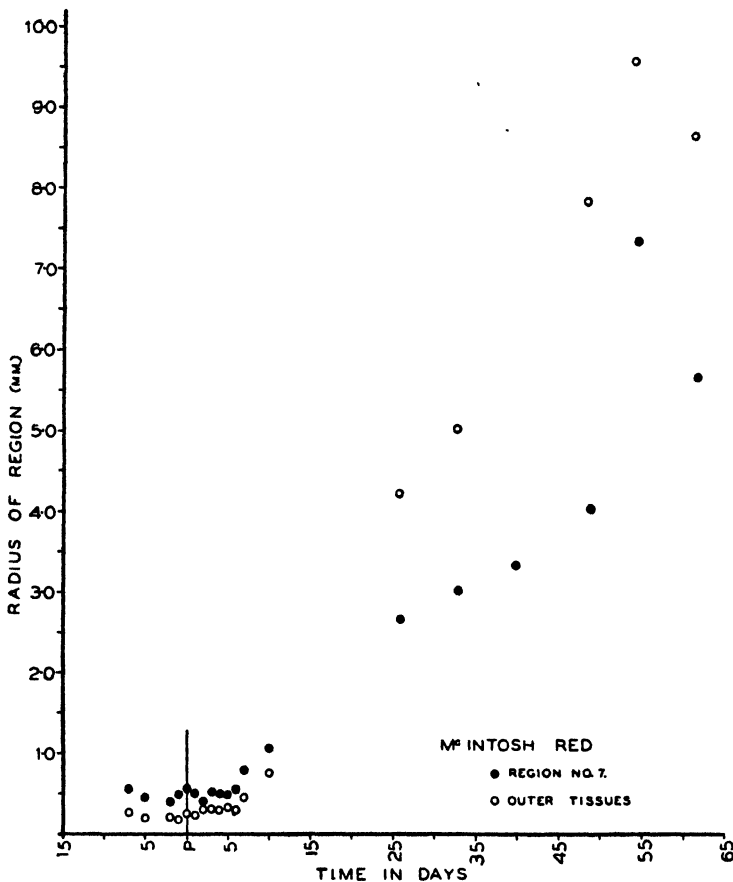


FIG. 3. Increase of radial extent of Region 7 and of the outer tissues (Regions 1 to 6 inclusive) with respect to time in the fruits of McIntosh Red. P = pollination.

random sampling of cells for measurement, and (b) the individual apples vary in size on the same date. However, the trends are indicated. Region 7, which is initiated by centripetal growth of the ventral carpel lobes (4), has a slightly greater radial extent at the time of anthesis. The slow period of growth which continues for several days after pollination is observable in both outer tissues and carpel regions. It is not as clear as in the composite graph (Fig. 2). In the McIntosh the radial extent of the outer tissues surpasses that of Region 7 at an early date. This is probably due to (a) the low set of seeds in the self-pollinated McIntosh, and (b) the "open core" condition. Direct observation reveals that the ventral bundles of those carpels with abortive ovules do not increase appreciably in diameter, whereas the bundles of those with developing ovules do, a feature that is very obvious when both types appear in the same fruit. An associated effect is the limiting of the extent of growth in the tissues external to unfertilized carpels and the increasing of it in fertilized ones, with resultant eccentricity of shape. Moreover, the

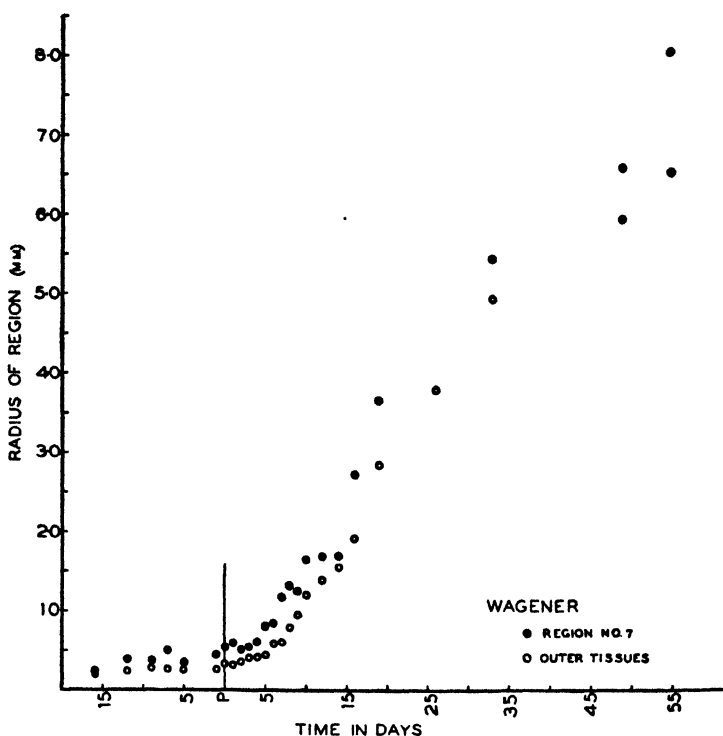


FIG. 4. Increase of radial extent of Region 7 and of the outer tissues (Regions 1 to 6 inclusive) with respect to time in the fruits of Wagener. *P* = pollination.

degree of eccentricity depends on the stage of development in the ovules when abortion occurs. For these reasons postpollination measurements are recorded only where ovules show signs of fertilization. The two regions in Wagener maintain a nearly constant rate of growth until the second week in July, or about five weeks after pollination. The scarcity of points on the graph beyond this date precludes definite conclusions, but comparison of this figure with that for the McIntosh (Fig. 3) indicates that growth of the outer tissues then surpasses that of Region 7. Moreover, this is coincident with that period in developmental history when activity in Regions 5 and 6 is apparent.

Each of the outer tissues, Regions 1 to 6, has a different mean cell dimension. Of these, the tissue having both the largest mean cell dimension and the greatest radial extent is in Region 4. In Fig. 5 the respective mean cell dimensions of Region 4 (cortex proper) and of Region 7 are plotted against the individual radial dimensions of that region. The lower part of the curve for Region 4 in the McIntosh shows a gradual increase in the mean size of the cell with an increase in the radius of the region. This is during the slow period of growth preceding and immediately following pollination, and apparently occurs before the effect of fertilization is impressed upon the tissue. The lower flattened portion of the curves for this region in the two varieties

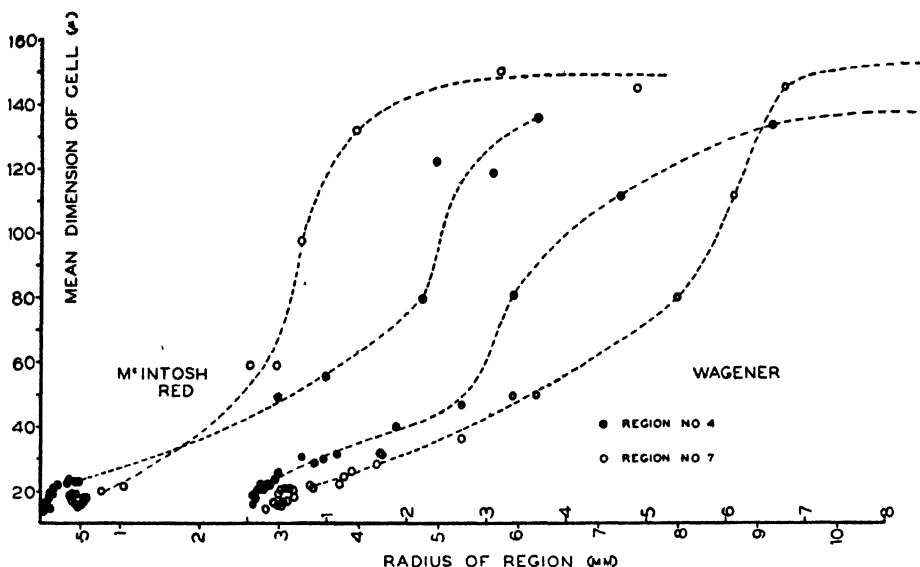


FIG. 5. Graphs of mean dimensions of cells in Regions 4 and 7, respectively, against radial extent of the regions in McIntosh Red and Wagener.

must indicate a period of cell division, since the radius of the region increases at a much greater rate than the mean size of the cell. In the McIntosh Red the period of cell division in Region 4 is more prolonged than in the Wagener, and is comparable to that in Region 7 in the latter. The more perpendicular segment of the curve is obviously the period of cell enlargement. Cell enlargement neither begins nor ends abruptly in either variety. Examination of the material discloses that before the steep segment of the curve, some of the nuclei are central, others are parietal. Scattered throughout the tissue are pairs of cells that are obviously the results of recent cell division. The upper portion of these curves, as cell size is again becoming a constant, represents a slight increase in mean cell dimension, and at the same time a slight increase in radial extent. Continued examination of sections indicates that this is not due to increase of cell number, but to the expansion of the intercellular spaces. In Region 4 of both varieties this dilation of intercellular spaces begins about nine weeks after pollination. Chemical tests for changes in the nature of the cell walls and more especially the cell contents might give some indication of the causes underlying the increase in intercellular volume. It is obvious that such tests should be made in the field where plenty of material at the proper stages is available.

The lower parts of the curves for both varieties indicate cell division. Little increase in cell size occurs at this time. The period of cell division for Region 7 in the McIntosh Red is similar to that for Region 4 in the Wagener. The radial extent of Region 7 in the Wagener is greater than that in the McIntosh before the rate of cell division diminishes. Cell enlargement follows, and succeeding that, the enlargement of the intercellular spaces, as in

Region 4. Cell size in Region 7 of the McIntosh very early exceeds cell size in Region 4. This does not occur in the Wagener until cell size in Region 4 has almost reached its maximum enlargement. Since there is no difference in the final cell size of comparable regions in the two varieties, and since the period of cell division in Region 7 of the Wagener is greater than that of the McIntosh, this region must contain a larger number of cells.

Fig. 6 is similar to Figs. 3 and 4. The outer tissues (Regions 1 to 6 inclusive) and Region 7, respectively, are plotted on a double log grid against the radii of the fruits. As this figure indicates, in both varieties the rate of increase of radial extent of the outer tissues is slightly higher than the rate of increase for the radius as a whole, and is also constant for the two varieties. Region 7 in the McIntosh has the same rate of increase of radial extent as the rate

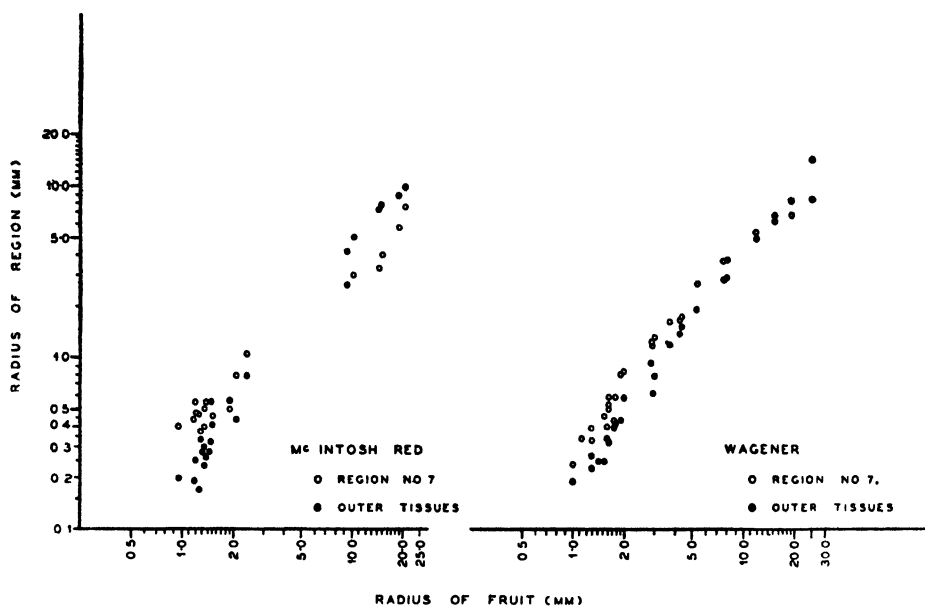


FIG. 6. Double log grid plot of radii of Region 7 and outer tissues (Regions 1 to 6 inclusive), respectively, against radius of the fruit in McIntosh Red and Wagener.

for the radius as a whole. On the other hand the same region in the Wagener has a higher rate than the radius as a whole for a time. This higher rate is equal to that of the outer tissues. When the regions are increasing by the enlargement of intercellular spaces, rate in Region 7 becomes lower than that for the radius as a whole.

Discussion

An evaluation of the data just presented on the trends in the development of the apple fruit of the varieties studied demands a discussion of the effective roles of the following: (1) cell division and cell enlargement, (2) the direction and extent of localized and general growth, (3) differentiation and organization

of the cells into tissues, and (4) the relative importance of these tissues in the production of the mature fruit. It is obvious that the mature fruit is not the result of the consecutive sequence of the above activities but of their integration.

Before anthesis there is a rapid development of the ovules, while the remaining tissues of the fruits increase but slightly in extent. After pollination there is a short period in which there is no significant change in the size of the fruit as a whole. This is more clearly observable in the McIntosh (Fig. 2), and may be due to the low degree of self-compatibility of the fruit. The Wagener shows the effect of fertilization (Fig. 2) earlier than the McIntosh as indicated by the measurable increase in size.

The initial effect of fertilization manifests itself in an impetus given to further cell division (Fig. 5). An examination of Fig. 2 shows that the radius of the fruit suddenly begins to increase rapidly five to six days after pollination.

A study of the early developing fruits indicates that these cell divisions are general and diffuse, and that they are periclinal in orientation. The potential fruit, until postfertilization growth alters it, is somewhat ellipsoidal without a cavity at the pedicel end. There is an apical depression or basin within and around which were earlier located the free floral parts. Cell multiplication continues for different periods of time not only in the several regions of the same fruit but in comparable regions of the two varieties. A comparison of Regions 4 and 7 in Fig. 5 illustrates the first condition, a comparison of the carpellary regions (Region 7) in the two varieties, the second. In all cases the period of general cell division is over before the end of June. After this time occasional cell divisions occur sporadically throughout the regions but further increase in diameter is essentially due to cell enlargement. As with cell division, cell enlargement neither begins nor ends abruptly. The grand period of cell enlargement differs in the various regions of the same fruit and in comparable regions of the two varieties (Fig. 5). During this period, the expansion of the cell in the three planes changes the shape of the fruit from ellipsoidal to subspherical. If the enlargement of the cells in all planes were equal and no other factors entered in, a median transverse section would have the outline of a perfect circle. This period of cell enlargement comes to a gradual end in the latter part of July (Fig. 5). As this cell enlargement slows down, there occurs a rapid increase in the size of the intercellular spaces, as evidenced by the upper flattened portions of the curves in Fig. 5. From this time on further increase in volume is primarily due to this phenomenon.

If this phenomenon were equally effective in all planes and in all regions the resulting mature fruit would be the continued expansion of the sphere, and a median transverse section would merely outline a larger circle than one of an earlier date.

One of the characteristics of the Wagener fruit is its angulation, whereas the McIntosh is only faintly angled. This angulation is caused primarily by (a) the original degree of fluting in the outline pattern of the primary vascular bundles (4), and (b) a localization of growth. After the period of general cell division, the cambia of the sepal and petal bundles become active in cutting off parenchyma cells that are added to the ground tissue in Regions 5 and 6. These cells enlarge and are oriented in a radial direction. Since the cambia of these bundles in the Wagener are more active than in the McIntosh, the original angulation is accentuated. Thus there is impressed on the fundamental pattern a localization of growth that changes the shape during ontogeny.

Cells at the apex of the pedicel and around the basin are laid down during the period of cell division. As the fruit develops and the cells of these regions enlarge in the three planes, the tissue grows down and out to form the conical cavity around the pedicel, up and out to form the enlarged basin. The cavity and basin are, therefore, in considerable measure at least, the results of the mechanics of cell enlargement.

Ultimate cell size and shape are the same in comparable regions of the two varieties. The duration of cell division varies with the region and with the variety. Therefore, final regional differences between the varieties must be due to differences in cell number.

The cellular pattern of development in the apple fruit, as portrayed in the McIntosh and Wagener varieties, is laid down early in ontogeny. Later, through the operation of different physiological factors, there is added to this fundamental pattern those differences of persistence, rates, and directions of localized growth in specific tissues. The integrated activities of cell division and cell enlargement and these localized growths together with the development of an intercellular system, result in the final shape and size of the mature fruit.

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FURTHER STUDIES ON CHANGES OF DIRECTION IN THE MAJOR COIL OF THE CHROMONEMA OF *TRILLIUM ERECTUM* L.¹

BY G. B. WILSON² AND ISABEL HUTCHESON³

Abstract

A detailed study of the direction of chromonema coiling at first meiotic metaphase and anaphase in *Trillium erectum* L. has shown that reversals in direction of coiling occur in the attachment region, at chiasmata, and in other regions. They occur with random frequency at the attachment. There is an average of two reversals at each "effective" chiasma. In other regions reversals were observed with a frequency of one in 17 gyres.

The results indicate that there is no inherent pattern determining the direction of coiling and that reversals are effected by various interrupting mechanisms.

Introduction

Any mechanism postulated to explain spiralization must meet the test of whether or not the spiral it would produce would have a directional pattern in agreement with that observed. It is, therefore, an essential part of the general problem of spiralization to determine the coiling pattern in as wide a variety of organisms as possible. So far the most detailed studies have been made on *Tradescantia*, by Nebel (9), Nebel and Ruttle (12), and Sax and Humphrey (14), on *Fritillaria*, by Darlington (1, 2, 3), and on *Trillium*, by Matsuura (7 and 8), Huskins and Smith (5), and Huskins and Wilson (6). Unfortunately the results of these studies are not in complete agreement. Darlington has stated that the arms of a chromosome almost always coil in opposite directions whereas most other workers have reported either that the direction of coiling is random or that it tends to be the same in both arms. Darlington and his co-workers have so rarely found reversals within a chromosome arm that they are inclined to consider them as relatively unimportant to general theory (16). On the other hand, workers on *Trillium* (especially Matsuura, and Huskins and his co-workers) have found many intrabrachial reversals in the major coil of meiosis. In *Tradescantia* and *Rhoeo* (12 and 13), the number of reversals per chromosome is apparently small. The present investigation has been undertaken in the hope of finding at least a partial solution to the problem of the frequency and distribution of reversals by a more detailed study of the major coil of *Trillium erectum* L. than has previously been made.

From their studies on synaptic, desynaptic, and asynaptic *Trillium erectum*, Huskins and Wilson (6) concluded that reversals in the major coil were probably of random occurrence at the attachment and at chiasmata and that, in addition, reversals could occur at any point along the chromosome with a

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frequency proportional to the number of gyres. They based their conclusion on an analysis of the total number of reversals at first anaphase at which stage it is easiest to trace the coils throughout the length of the chromonema. In the present study, observations have been made at both first metaphase and anaphase.

Materials and Methods

Most of the data on synaptic material presented in this paper have been obtained from a single slide that contained both first metaphase and anaphase stages of *Trillium erectum*. This slide was chosen because of the relative ease with which all four strands could be distinguished at metaphase (Plate I). Data on desynaptic material have been obtained from the slide used by Huskins and Wilson (6).

Fixation was in 2BD and staining in crystal violet as described by Huskins and Smith (5). Observations were made with a Zeiss 1.5 mm., 1 3 N.A. objective, combined with 7 \times and 15 \times oculars. A 3 mm., 1 4 N.A. objective and 7 \times ocular were used when photomicrographs were taken.

In order to avoid the possibility of confusion several of the terms as they are used herein will be defined.

Interbrachial reversals. Changes in the direction of coiling that occur in the attachment region.

Intrabrachial reversals. Changes in the direction of coiling that occur within a chromosome arm.

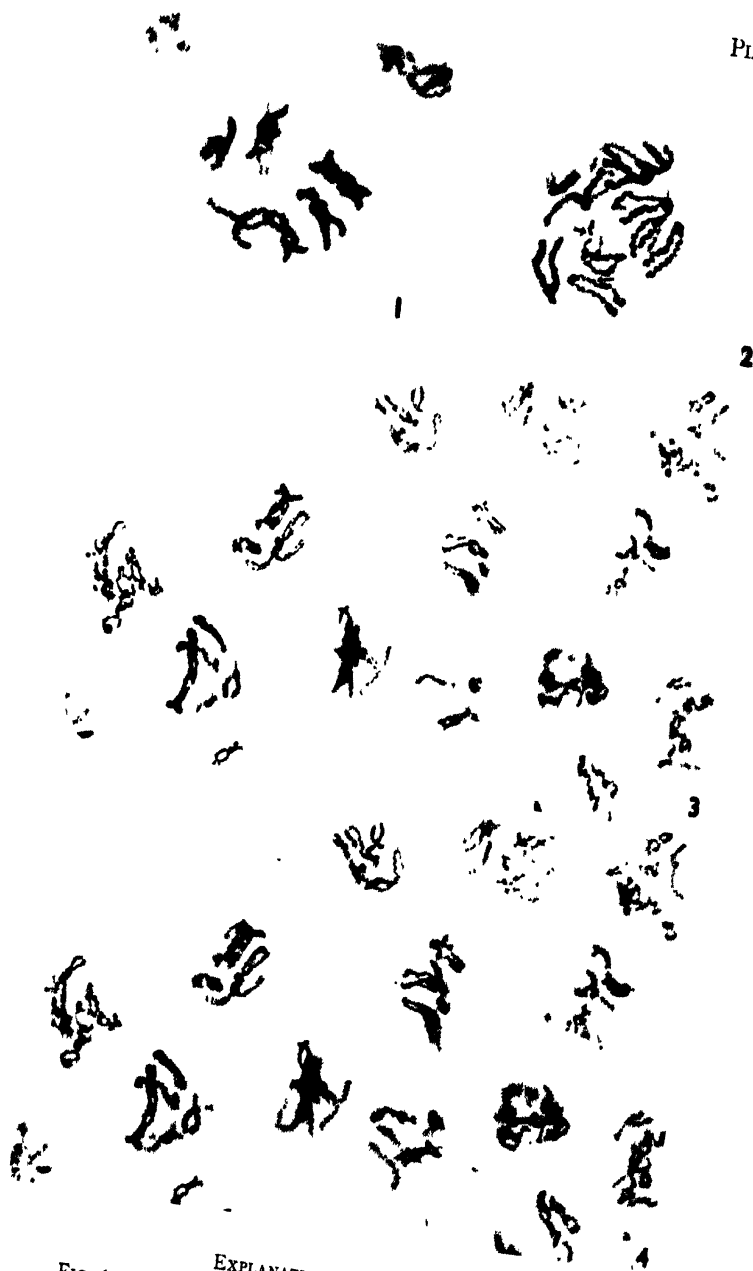
Adventitious reversals. Those intrabrachial changes of direction not directly due to the exchange of partners at a chiasma.

True chiasma frequency. The total number of chiasmata per complement at first metaphase, determined from preparations in which all four strands can be distinguished clearly.

Effective chiasma frequency. The number of chiasmata per complement at first metaphase when those lying between the attachment and the proximal gyre are deducted and pairs of chiasmata separated by less than one gyre are counted as one chiasma (see also (15)). This distinction is based on the assumption, at least partly justified by observations, that any effect that chiasmata may have on coiling is purely mechanical and that two chiasmata if close together behave as a unit in this regard.

"Sister" strands. The two chromatids that are most closely paired at metaphase; these are doubtless genetic sister strands before movement of chiasmata, but not necessarily afterwards.

Chiasma region. The region in the vicinity of a chiasma in which the four strands are drawn out of their usual paired arrangement by the exchange of partners.



EXPLANATION OF FIGURES

FIG. 1. First metaphase. Coiling is nearly completed. 750 \times .

FIG. 2. First anaphase. Note reversals in direction. 750 \times .

FIGS. 3 AND 4. Two foci of the same region showing the different stages in the development of the major coil. Coiling is just beginning in the cell at the extreme left. ca. 550 \times .

Observations

(1) *Frequency of Reversals at the Attachment Region*

Huskins and Wilson (6) assumed that reversals in the direction of coiling occurred with random frequency at the attachment. They based this conclusion largely on the results obtained from asynaptic material since in this the chromatids are separated through the attachment region and there are no chiasmata to complicate the situation. Dr. A. W. S. Hunter (unpublished data) had previously found that in this material 18 out of 41 chromatids reversed direction at the attachment. While it is reasonable to assume that the effect of the attachment on direction of coiling would be similar in synaptic and asynaptic materials, this has not previously been established.

In synaptic material the effect on coiling of the attachment alone cannot readily be ascertained since the majority of bivalents have chiasmata proximal to the first gyre and they themselves have an effect on the direction of coiling. For this reason the present study has been confined to a determination of the effect of the "attachment region" on coiling direction. The "attachment region" may consist of the attachment alone, or it may also include one or two chiasmata.

A second difficulty encountered in synaptic material is that in the majority of cases the chromatids cannot be traced through the attachment. When "sister" chromatids are both coiling in the same direction on at least one side

TABLE I
AN ANALYSIS OF THE FREQUENCY OF REVERSALS IN THE
ATTACHMENT REGION

Analysis	Number of reversals	
	0 or 2	1
Dyad		
First metaphase		
One chiasma	15	11
Two chiasmata	18	14
First anaphase	45	55
Total	78	80
Chromatid	0	1
Synaptic		
First metaphase		
No chiasmata	8	8
One chiasma	30	22
Second anaphase	14	12
Subtotals	52	42
Desynaptic	54	46
Asynaptic (Hunter)	23	18
Totals	129	106

of the attachment region there is no difficulty in making an accurate count of chromatid reversals since it makes no difference in this case whether the strands can be traced independently through the attachment or not. However, when the strands associated at the attachment become separated by chiasmata and coil in opposite directions to each other on both sides of the attachment it is impossible to determine whether no reversals or two reversals have occurred if the strands cannot be traced independently (e.g., a dyad or half-bivalent, $\begin{smallmatrix} R & R \\ L & L \end{smallmatrix}$ o $\begin{smallmatrix} R & R \\ L & L \end{smallmatrix}$, may actually have either 0 reversals or 2). On a random basis dyads with 0 reversals, 1 reversal, and 2 reversals would occur in a ratio of 1 : 2 : 1. Since it is not always possible to separate the 0 and 2 reversal classes they will be grouped together and tested for equality with the reversal class.

The frequency of reversals at the attachment region is given in Table I, and it was found that 78 dyads had either 0 or 2 reversals, and 80 dyads had one.

Unless the individual chromatids can be traced through the attachment region analyses cannot be made in terms of single chromatids at metaphase for bivalents with chiasmata between the attachment and the first gyre in both arms nor for dyads at first anaphase. But even if the strands cannot be traced through the attachment, since "sister" strands coil together in normal synaptic material except in the neighbourhood of chiasmata, chromatid analyses of coiling can be made when there is no chiasma between the attachment and the first gyre on either or both sides of it. The chromatid analyses given in Table I show that 42 chromatids had reversals at the attachment region and 52 did not. This number is well within the limits of random expectation ($\chi^2 = 1.06$, $p = 0.3 - 0.5$).

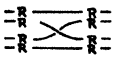
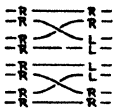
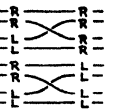
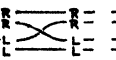
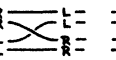
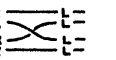
In desynaptic material the chiasmata are mostly resolved before coiling begins and the relation of the attachment alone to coiling direction can therefore be analysed. Furthermore, the chromatids are well separated at the attachment and can be traced individually through it with certainty. Of the 100 chromatids studied 46 had a reversal at the attachment and 54 did not ($\chi^2 = 0.64$, $p = 0.6$).

Random expectation is therefore realized in all three tests.

(2) Frequency of Reversals at Chiasmata

In normal synaptic *Trillium erectum* a bivalent consists of two pairs of chromatids with the direction of coiling random between the pairs but the same within each except near chiasmata; there three of the four chromatids may coil in one direction and one in the other (5, 17, and Section 3). When homologues are coiled in opposite directions, the exchange of partners at chiasmata will result in reversals of coiling. In addition, adventitious reversals may occur within the chiasma region where all four strands are separate for a short distance. Combining these two sources of reversals, eight

configurations with respect to direction of coiling may occur at chiasmata (Text-fig. 1). If each of the four strands in a chiasma region is potentially capable of reversing direction with random frequency these configurations may be expected in equal numbers. These configurations represent 0, 2, 2, 2, 2, 2 and 4 chromatid reversals, respectively, so that 0, 2, and 4 change classes are expected in a ratio of 1 : 6 : 1. A sample of 100 chiasmata were analysed and the results are presented in Text-fig. 1. The numbers found (15 : 71 : 14) fit the expected ratio ($\chi^2 = 0.89$, $p = 0.5 - 0.7$).

Number of chromatid reversals	0	2				4
Configurations						
	15	46	15	10	14	
		71				

TEXT-FIG. 1. Coiling configurations at chiasmata.

(3) Frequency of Reversals in Other Regions

Huskins and Wilson (6) found a certain number of intrabrachial changes in direction at first anaphase in synaptic material which statistical analysis indicated were not associated with chiasmata. These were found to have a frequency proportional to chromonema length or gyre number. This analysis was supported by direct observations on asynaptic material where, also, intrabrachial changes were found to occur. In the present analysis first metaphase chromatids have been studied in detail in regions lying between successive chiasmata not less than four gyres apart, and in regions beyond the most distal chiasma. The numbers of changes occurring in these two regions were found to be similar, there being one change in 17.2 gyres in the former group and one change in 17.7 gyres in the latter.

To test in detail the conclusion of Wilson and Huskins (17) that the direction of coiling of the two pairs of chromatids of a bivalent is random with respect to each other, while the "sister" chromatids constituting a pair coil jointly, an analysis has been made, gyre by gyre, of the direction of coiling within and between the pairs of chromatids constituting a bivalent. The "between pairs" analysis showed 94 gyres in the same direction and 104 in the opposite direction. No sister chromatids were found to coil in opposite directions in those regions unaffected by chiasmata.

(4) Frequency of Intrabrachial Reversals at First Anaphase

First anaphase chromosomes have been analysed to determine the coiling pattern in whole complements of 20 chromatids. In this analysis the relationship between sister strands is disregarded. The observations were made on 10 cells from the same slide that was used for the first metaphase

analyses. The mean number of intrabrachial reversals per complement was found to be 35.9 ± 1.9 and the mean number of gyres 209.5 ± 1.9 (Table II). Though not pertinent to the present problem, it is of interest to note that there were 1126 dextral and 1069 sinistral gyres. The difference is not a significant deviation from equality.

TABLE II
NUMBER OF INTRABRACHIAL REVERSALS AND NUMBER OF
GYRES AT FIRST ANAPHASE

Cell	Number intrabrachial reversals	Number gyres
1	29	208
2	26	197
3	41	219
4	36	214
5	41	209
6	35	212
7	32	205
8	37	203
9	49	214
10	33	214
	359	2095
Mean	35.9 ± 1.9	209.5 ± 1.9

Discussion

A comparison of the behaviour of synaptic, desynaptic, and asynaptic materials has shown that, in all three, reversals take place at random in the attachment region. In the synaptic material one or two chiasmata are usually associated with the attachment, whereas, in the other two, no chiasmata are involved. Since there is such close agreement among the materials with regard to the effect of the "attachment region", it seems reasonable to assume that the attachment plus one or two associated chiasmata has the same effect on coiling as the attachment alone.

At chiasmata it has been shown that 0, 2, and 4 chromatids change in a ratio not statistically significant from the expected ratio of 1 : 6 : 1, indicating that adventitious reversals and those due to exchange of partner occur in equal numbers. Since the 0 and 4 change classes are equal, there must be an average of 2 reversals to a chiasma. It is, however, impossible to differentiate between reversals brought about by change of partner at a chiasma and those that occur through undefined causes in the unpaired region adjacent to it.

No significant difference was found between the number of adventitious reversals in regions between chiasmata and the number in regions distal to the last chiasma. In both cases changes occurred approximately once in

17 gyres. The undefined causes producing adventitious reversals must include the meeting of regions that have independently begun to coil in opposite directions, as they often clearly appear to do in *Trillium*. This may be a large factor in determining the reversals at chiasmata since these are obviously points of interference in the coiling process.

In their study on the direction of coiling, Huskins and Wilson (6) subdivided the total number of changes per nucleus observed at first anaphase into several groups. These groups were then used to reconstruct the hypothetical first metaphase bivalent in which changes in direction of coiling had earlier occurred. In the present study changes have been observed at first metaphase. The first anaphase configurations that would result from these have been predicted and compared with those observed at this later stage (Table III). There is close agreement between the calculated and the observed number of changes.

TABLE III
FREQUENCY OF INTRABRACHIAL REVERSALS AT FIRST ANAPHASE

Observed		Hypothetical				
Intrabrachial reversals per cell	Gyres	Intrabrachial reversals			Gyres*	
		Within chiasma regions**	In other regions	Total	Within chiasma regions	At other loci
35 9 ± 1 9	209 5 ± 1 9	31	5	36	125	85
Chiasma frequency						
True	Effective					
24.36 ± 0 58	15 6 ± 0 4					

* It has been assumed on the basis of the observations that each chiasma involves approximately eight gyres (two gyres per strand).

** Computed from the effective chiasma frequency.

Conclusions

While some details of the problem of the direction of coiling are yet to be investigated there seems little doubt from the results presented here together with those of Matsuura (8) and Huskins and Wilson (6) that the distribution of reversals in the direction of coiling is incompatible with torsion theories of spiralization. The fact that reversals occur with random frequency at the attachment region and at chiasmata and with a frequency proportional to length elsewhere indicates that there is no inherent structural pattern within the chromonema that determines direction of coiling or causes reversals.

Acknowledgments

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VEGETATIVE PROPAGATION OF CONIFERS

X. EFFECTS OF SEASON OF COLLECTION AND PROPAGATION MEDIA ON THE ROOTING OF NORWAY SPRUCE CUTTINGS¹

By J. L. FARRAR² AND N. H. GRACE³

Abstract

Twenty-four collections of Norway spruce cuttings were taken, seven about the time new growth was forming, six at semimonthly intervals from July to September, four during October, and seven at monthly intervals during the winter to April, and were propagated in outdoor frames in several media. The proportion of cuttings rooting in sand was low for the summer collections but reached 80% or higher for collections made in September and October. The addition of sedge peat effected rooting of 90% in collections taken throughout summer and autumn, and increased the number of roots, length of root, and the development of new growth. Sphagnum peat added to the sand was also slightly beneficial but, for propagation, much inferior in effect to the sedge type of peat. Varying the proportion of peat or the texture of the sand had no significant effect except on the length of root, which was greater in those media rich in peat. Cuttings stored over winter or taken in spring did not respond well.

Previous communications by the authors and others have dealt with the vegetative propagation of conifers (1-13). A recent article summarized the effects of chemical treatments on the outdoor propagation of Norway spruce cuttings (11). In this communication is reported the result of a series of experiments directed to the outdoor propagation of Norway spruce cuttings taken at various seasons and propagated in different media.

Experimental

This investigation of the effects of developmental stage, for convenience designated period of collection, and media on propagation involved 10 experiments and approximately 10,000 cuttings; only six of the experiments will be discussed in detail, the results from the others will merely be summarized. Most of the experiments were of factorial design permitting consideration of other factors such as chemical treatments or type of cutting in conjunction with effects of media and collection. The general methods of collecting and planting the cuttings have been described (11, 12).

Three different types of media were used, namely, sand, sand mixed with an imported peat of sphagnum origin, and sand mixed with a domestic, well decomposed peat of sedge origin* (4, 12). Data concerning chemical analyses of the two different peats are given in Table I. It is apparent that the sedge type of peat has a substantially greater content of both acid soluble and

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* The sphagnum peat, from Sweden, was obtained through a local horticultural supply house; the sedge peat was obtained directly from Alfred, Ont.

TABLE I
CHEMICAL ANALYSES OF SEDGE AND SPHAGNUM PEAT

	Composition, %	
	Sedge peat	Sphagnum peat
Loss on ignition	80.6	95.0
Containing nitrogen	2.12	0.94
Mineral matter insoluble in acid	13.4	3.5
Mineral matter soluble in acid	6.0	1.5
Containing: lime	2.16	0.40
potash	0.06	0.09
phosphoric acid	0.23	0.23
Acidity (pH)	5.6	4.0

The authors are indebted to the Division of Chemistry, Central Experimental Farm, for the analyses.

insoluble minerals and more nitrogen; it is also less acidic. Though the chemical properties of these peats are different, their physical characteristics are essentially similar. The usual moisture content of media containing one-third peat by volume was about 14% of the oven-dry weight whereas that of sand alone was about 4%. The sand media and sand-peat mixtures involved the use of two different types of sand, one relatively coarse, the other fine; screen analyses of these sands are given in Table II. Equal proportions of these sands were used in the sand media of Experiments 1, 2, and 3 and in the peat mixtures of Experiments 1, 2, 3, 5, and 6 and several proportions of each as described under Experiment 4; fine sand alone mixed with an equal amount of peat was also used for Experiments 5 and 6.

TABLE II
SCREEN ANALYSES OF SANDS USED AS MEDIA

Mesh, openings to the inch	Coarse sand		Fine sand	
	Retained, %	Passed, %	Retained, %	Passed, %
4	1.0	99.0	1.3	98.7
8	4.4	95.6	2.9	97.1
10	12.8	87.2	4.2	95.8
20	56.6	43.4	8.9	91.1
35	92.5	7.5	28.6	71.4
65	99.2	0.8	77.5	22.5
100	99.7	0.3	90.8	9.2

Experiment 1

Cuttings were collected at semimonthly intervals during July and August, 1939, and planted in three media. These were sand, sand mixed with one-third by volume of sphagnum peat, and sand with one-third by volume of sedge peat.

Experiment 2

In this experiment the semimonthly collections of Experiment 1 were continued throughout September and October. Media were reduced to two by eliminating the sphagnum peat mixture.

Experiment 3

Cuttings were collected October 29, 1939, and planted in the two peat media described under Experiment 1.

Experiment 4

Cuttings were collected October 29, 1939, and planted in 28 different media in compartments 1 × 3.5 ft. in size. Sand was mixed with sphagnum and sedge peat, each separately, in proportions by volume of eight sand to one, two, four, and eight of peat. The mixture of eight sand to four peat was identical with the media used in the preceding experiments. Three different grades of sand were used to make the various media; these were the two described in Table II, and a mixture of the two in equal proportions. In addition to 24 mixtures of sand and peat there were four sand media—one volume coarse sand with one, two, and four volumes of fine sand, and fine sand alone.

Experiment 5

The branches were collected approximately every four weeks starting November 17, 1939, with the last of the seven collections April 23, 1941. Cuttings were made and heeled in in flats in a sand-peat mixture and kept outdoors protected with a mulch of straw. In May the cuttings were planted in two media, the sedge peat mixture of Experiments 1, 2, and 3 and fine sand with an equal volume of sedge peat. At the time of the last collection cuttings were also taken from branches of the first collection, which had been stored outside during the winter.

Experiment 6

Collections were made June 6, 1940, after the new shoots were about one inch in length and June 27 when elongation of new growth on the lateral branches had ceased. Cuttings of this second collection were entirely of 1940 growth. Both collections were propagated in the media of Experiment 5.

A group of experiments was started in the spring of 1939. These involved collections about two weeks prior to the emergence of new growth, just before emergence, during the opening of the buds and after the shoots had appeared. In addition, there were collections in which the cuttings were entirely of 1939 growth. The cuttings for all these experiments were propagated in sand only.

It has also been possible to develop some additional criteria from the results of a greenhouse experiment already reported by Farrar (3) and Deuber and Farrar (2). The original papers dealt chiefly with effects on rooting; the additional results now considered refer to effects of time of collection on the development of new growth by the cuttings and the numbers and lengths of root per rooted cutting.

The cuttings of Experiments 1 to 6 were removed for observation in September, 1940. Record was made of the number of cuttings surviving, callused, rooted, and the number and length of roots per group of cuttings. The number and length of roots per rooted cutting and the mean root length were calculated. Data were recorded separately for cuttings with and without new growth. The number of new growth shoots per group of cuttings and the aggregate length of the longest shoot were determined for rooted and not rooted cuttings. Statistical treatment of similar data has been described in earlier articles (3, 5-13).

Results

Table III indicates the effects of the propagation medium and season of collection on responses of Norway spruce cuttings. The data are averages over other experimental factors such as chemical treatment, type of cutting, and proportions of peat and sand in the medium when these factors were without significant effects.

It is apparent from the data of the table that cuttings collected between mid-July and late October and propagated in a sedge peat medium responded favourably. Those planted in sand or sphagnum peat media, or in a sedge peat medium at other times of the year did not respond so well.

The season of collection had little effect on the rooting of cuttings planted in sedge peat from mid-July to late October. On the average, over 90% of the cuttings were rooted each with four to five roots averaging about 5 cm. in length. The best individual group of 10 cuttings is illustrated in Fig. 1. The percentages of winter and spring collections rooting was not as high and the average number of roots was substantially lower. However, the mean root length did not differ greatly in the winter collections, though it was much shorter in the spring collection (Experiment 6). These cuttings were, of course, in the beds for a shorter period than the previous collection. In both the sand and sand-sphagnum-peat media there were many living cuttings that failed to root and mortality was markedly greater than in the sedge peat media. The proportion of living non-rooted cuttings was particularly high at the period of low rooting and survival was also low at these periods.


The proportion of cuttings with new growth was approximately the same among rooted cuttings and those living but not rooted and it is apparent that the presence of new growth on a cutting was no indication that it was necessarily rooted. Under the more favourable conditions of media and collection (plantings in sedge peat media and summer collections) most of the rooted cuttings bore new growth, while under less favourable conditions most of the rooted cuttings lacked such growth. (In the collection of June 6, Experiment 6, all cuttings had new growth from the beginning.) The effect of media and season of collection on the production of new growth shoots was similar to the effects on survival and rooting.



1

FIG. 1 Norway spruce cuttings propagated outdoors in a sedge peat medium, the best individual group of 10 cuttings (Background ruled in inches.)



 A large proportion of the cuttings planted in sedge peat media during the summer produced new growth shoots the following spring. In sand only, few cuttings produced new growth but the proportion increased markedly with the later collections. Most of the cuttings with new growth bore only one shoot but the summer collections in sedge peat media resulted in a greater number and length of shoots than was found for the other two types of media or other times of collection. In the first collection of Experiment 6 new growth was produced while the cutting was still on the tree, and on the average, there were finally two shoots per cutting.

The data on numbers and lengths of roots and the proportion of surviving cuttings that rooted were analysed separately for cuttings with and without new growth. It was found that the results were essentially similar for both classes of cuttings. Data on numbers and lengths of new growth shoots were analysed separately for cuttings with and without roots and it was again shown that the results for both classes of cutting were essentially similar.

An outstanding feature of the results is the difference between sphagnum and sedge peats. Reference to the data for Experiments 3 and 4, taken at probably the optimum season for the outdoor propagation of Norway spruce cuttings, shows that in sedge peat the percentage rooting and length of root per rooted cutting and the number with new growth was approximately twice or more that obtained in sphagnum peat, and there were more and longer shoots. Typical differences between the effects of the two peat media on rooting and development of new growth are illustrated in Fig. 2.

The different proportions of peat used in Experiment 4 affected only the length of root per rooted cutting. There was a greater length of root associated with an increasing proportion of peat. Likewise the different proportions of sand made no significant difference to any of the factors used as criteria. The poor results in sand in Experiment 4 as compared with a similar collection in Experiment 2 may be attributed to deficient water supply. In Experiment 4 the compartments of sand were interspersed at random with compartments of peat media, which require less water; since the bed was watered as a whole the sand did not receive an adequate supply.

It is apparent from the data for Experiment 5 that cuttings made and planted in April (Collection 7) gave better survival and rooting than those made in November, which are, again, better than cuttings taken at the same time but stored over winter on the branch. However, about 82% of the surviving cuttings of all three of these collections were rooted.

Spring collections made in 1939, and planted outdoors in sand, resulted in rooting of 10 to 25% of the cuttings taken in May about two weeks prior to emergence of new growth; this proportion rose to about 40% for those cuttings taken four days prior to emergence of new growth, and 75% for those in which new growth was breaking. About 64% of cuttings of 1938 wood bearing shoots ranging in length from 0.5 to 1 cm. rooted (11), and 45% of those with shoots from 1 to 2 cm. in length. The mortality of cuttings con-

TABLE III

RESPONSES OF NORWAY SPRUCE CUTTINGS TO PROPAGATION MEDIUM AND SEASON OF COLLECTION
(CUTTINGS WERE REMOVED SEPTEMBER, 1940)

Expt. No.	Collection date	Criterion								
		Number of surviving cuttings, %			Number of rooted cuttings, %			Number of cuttings rooted as percentage of those surviving		
		Medium								
		Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat
1	1939 July 12 July 26 Aug. 9 Aug. 23	31 31 13 15	78 94 78 62	96 100 100 98	26 16 8 5	70 76 56 32	90 98 98 82	84 52 62 33	90 81 72 52	94 98 98 84
2	Sept. 15 Sept. 29 Oct. 13 Oct. 27	78 97 92 100		98 99 97 99	56 86 81 95		90 97 96 98	72 89 89 95		92 98 99 99
3	Oct. 29		65	94		40	90		61	96
4	Oct. 29	75	82	97	54	56	93	73	69	96
5	Nov. 17 Nov. 17† Dec. 11 1940 Jan. 8 Feb. 2 Feb. 21 Mar. 27 April 23			57 40 67 53 46 52 70 95			47 33 49 33 29 38 44 78			82 82 74 61 64 73 63 83
6	June 6 June 27			74 52			37 26			49 49
		Criterion								
		Number of roots per rooted cutting			Length of roots per rooted cutting, mm.			Mean root length, mm.		
		Medium								
		Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat
1	1939 July 12 July 26 Aug. 9 Aug. 23	4 0 2 9 3 5 2 6	4 3 3 8 3 3 2 7	5 3 4 9 4 3 4 2	129 53 90 17	237 143 114 33	276 248 234 109	32 18 26 6	55 38 35 12	52 50 54 26
2	Sept. 15 Sept. 29 Oct. 13 Oct. 27	3 7 3 7 4 0 4 6		4 4 4 4 5 0 4 6	80 81 86 164		172 206 268 287	22 22 22 35		39 47 54 62
3	Oct. 29		3 3	4 2		127	225		39	54
4	Oct. 29	3 0	3 1	4.3	76	130	260	26	42	60
5	Nov. 17 Nov. 17† Dec. 11 1940 Jan. 8 Feb. 2 Feb. 21 Mar. 27 April 23			3 0 2.5 2 8 1.9 2 1 2 7 2 9 3 3			145 145 133 103 99 133 153 178			48 59 47 55 47 49 53 55
6	June 6 June 27	-		2.2 2 1			28 14			13 7

† The cuttings of this collection were made April 23, 1940, from branches held outside in storage from November 17, 1939.

TABLE III—*Concluded*RESPONSES OF NORWAY SPRUCE CUTTINGS TO PROPAGATION MEDIUM AND SEASON OF COLLECTION
(CUTTINGS WERE REMOVED SEPTEMBER, 1940)—*Concluded*

Expt. No.	Collection date	Criterion								
		Number of cuttings with new growth, %			Number of cuttings with new growth as a percentage of those surviving			Number of rooted cuttings with new growth as a percentage of those rooted		
		Medium								
		Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat
1	1939 July 12 July 26 Aug. 9 Aug. 23	2 0 0 5	4 0 2 4	64 54 38 58	6 0 0 33	5 0 3 6	67 54 38 59	8 0 0 4	6 0 4 12	69 55 37 63
2	Sept. 15 Sept. 29 Oct. 13 Oct. 27	23 57 39 60		61 66 68 73	29 59 42 60		62 67 70 74	30 57 46 60		62 68 71 73
3	Oct. 29		12	70		19	75		24	75
4	Oct. 29	31	28	75	42	34	77	43	34	77
5	Nov. 17 Nov. 17† Dec. 11 1940 Jan. 8 Feb. 2 Feb. 21 Mar. 27 April 23			7 3 15 7 4 14 10 37			12 8 23 12 9 27 14 39			12 6 25 13 • 25 17 43
6	June 6 June 27			74 0			100 0			100 0

		Criterion								
		Number of rooted cuttings with new growth as a percentage of those with new growth			Number of shoots per cutting with new shoots			Mean length of longest shoot on each cutting with new shoots, mm.		
		Medium								
		Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat
1	1939 July 12 July 26 Aug. 9 Aug. 23	• • • •	• • • •	97 100 95 90	• • • •	• • • •	1.59 1.33 1.32 1.21	• • • •	• • • •	22 18 18 25
2	Sept. 15 Sept. 29 Oct. 13 Oct. 27	74 86 95 95		92 100 100 99	1.13 1.28 1.10 1.22		1.38 1.39 1.34 1.45	18 20 17 20		29 25 24 33
3	Oct. 29		79	95		1.10	1.36		18	35
4	Oct. 29	74	69	95	1.14	1.10	1.39	16	17	27
5	Nov. 17 Nov. 17† Dec. 11 1940 Jan. 8 Feb. 2 Feb. 21 Mar. 27 April 23			76 62 81 63 • 68 75 90			1.00 1.12 1.24 1.12 1.20 1.12 1.33 1.24			20 24 27 18 17 25 20 22
6	June 6 June 27			49 ?			2.06			24

† The cuttings of this collection were made April 23, 1940, from branches held outside in storage from November 17, 1939.

‡ Meagre data.

sisting entirely of 1939 growth planted in sand in early June was 100%. In cuttings of 1939 wood with new growth planted in sand peat in 1940, 37% rooted while the proportion of new growth cuttings rooted was 26% (Table III, Experiment 6).

Counts of the number of cuttings with new growth and the numbers and lengths of root per rooted cutting for winter collections of cuttings propagated in sand in the greenhouse at New Haven indicated marked effects from season of collection. Data were meagre for cuttings of the October collection, those of the following three months had 2.6, 4.1, and 3.2 roots per rooted cutting, respectively, and the corresponding lengths of root were 32, 121, and 94 mm. Thus, December and January collections produced more and longer roots than the one in November. Even more striking differences were revealed by the counts of new growth which were approximately 80 and 90%, respectively, for December and January collections but less than 1% for October and November collections.

Discussion

These results have demonstrated the great effect of medium on the rooting of summer and autumn collections of Norway spruce cuttings propagated outdoors, and are similar in nature to earlier results for fall and winter collections (4, 10-12). When a suitable medium such as well decomposed sedge peat is used, effects attributable to the period of collection are slight. However, when a much less suitable medium such as sand only is employed, period of collection of the cuttings may have a pronounced effect on the results.

The authors have already discussed changes in rooting response with period of collection (3, 4, 7, 9) and recently, Deuber has presented such data also for Norway spruce cuttings propagated in sand in the greenhouse (1). The present experiments indicate certain modifications of the earlier views. The percentages of rooted cuttings of new growth, taken when elongation of the lateral twigs has ceased, were low. Three weeks later about 90% of the cuttings planted in a sedge peat medium were rooted and this level was maintained until freeze-up. Cuttings planted in sand did not root well until September, from then on the rooting percentage was almost as high as in peat though numbers and lengths of roots were lower.

After the ground is frozen cuttings must either be planted in a greenhouse or stored till spring. The results of Experiment 5 indicate that the latter practice substantially reduces rooting. This may be attributed to unfavourable storage conditions and injury in transplanting. The rise in rooting from February to March could be explained in part by the shorter storage period. The decline in rooting from November to February is very similar to that observed in the greenhouse by Deuber and the authors (1, 7, 9). It seems likely that the decline in the rooting potential at this period is due to physiological changes in the tree.

Results have been variable with cuttings taken before and during the development of new growth. There is evidence that cuttings taken just as the buds are breaking root more readily than cuttings taken just before or just after this time.

These experiments indicate that bud development and rooting occur independently. However, conditions favourable to the one also favour the other.

The fact that the beneficial effect of sedge peat in sand mixtures is, within wide limits, unaffected by the proportion of peat in the texture of the sand, greatly simplifies its use in practice.

Norway spruce cuttings taken during summer or autumn can be propagated outdoors in readily constructed frames by the use of sedge peat mixed with the sand. This finding is of particular interest to tree breeders and horticulturists who may wish to propagate certain individuals of this species.

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STUDIES IN DIFFERENTIAL REACTIVITY

I. THE RATE AND DEGREE OF DIFFERENTIATION IN THE SOMATIC CHROMOSOMES OF *TRILLIUM ERECTUM* L.¹

BY G. B. WILSON² AND E. ROGER BOOTHROYD³

Abstract

By suitable treatment the somatic chromosomes of a number of plants may be shown to be longitudinally differentiated as to size and staining capacity. Certain well defined regions appear at metaphase and anaphase to be either understained or of reduced diameter or both.

Exposure to cold has been found to produce these regions in two varieties of rye and three species of *Trillium*.

The positions of these differential segments have been found to be highly specific for different chromosomes and species.

During a 96-hr. period of exposure to cold the number of chromosomes affected and of differential segments per chromosome increases in *T. erectum* L.

After cessation of treatment the chromosomes resume their normal appearance within a few hours.

Homologous chromosome pairs often differ in number, size, and position of affected regions. On the basis of data presented in this paper, these differences cannot be taken as conclusive evidence that these pairs are genetically different.

Introduction

From time to time there have appeared in the literature accounts of experimental treatments that rendered visible a differentiation of the chromosomes not otherwise observable. This differentiation has taken the form of regions of reduced diameter and staining capacity and of secondary constrictions, sometimes so numerous as to give the chromosomes a chromomeric appearance. The former may be of considerable length and may appear either within or at the ends of chromosome arms.

Kagawa (9, 10) and Ellenhorn (4) have shown that pretreatment of *Triticum* root tips with chloral hydrate before fixation renders visible certain specifically localized secondary constrictions that cannot be seen without such pretreatment. Similar results have also been obtained in the somatic chromosomes of *Trillium smallii* Maxim. and *T. Tschonskii* Maxim. (8).

Geitler (7) observed "chromomeres" in the somatic metaphase chromosomes of *Crepis capillaris* Wallr. after fixation with the Flemming-Benda mixture. Similar results were obtained, according to Shmargon (12) in *Allium cepa* L. by growing the plants in sawdust, and fixing with Champy's fluid. One chromosome contained over 20 "chromomeres" well differentiated in size.

Kakhidze (11) confirmed Geitler's observations in *Crepis*, finding the metaphase chromosomes uniformly divided into "chromomeres". When the roots

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were precooled, however, secondary articulations that were apparently quite distinct from the "chromomeres" appeared in each arm.

Shmargon (12) observed a "chromomeric structure" in the mitotic chromosomes of *Secale cereale* L. after precooling the material (24 hr. at 0° C.) and using Levitsky's "strong platinic formalin" and Champy's fixatives. He believes these "compound chromomeres" to be formed by fusion of the "ultimate chromomeres".

Darlington and La Cour (2, 3) found understained regions of reduced diameter, and highly specific as to locality, in mitotic metaphase and anaphase chromosomes of *Paris* and *Trillium* subjected to cold treatment for several days. They called this phenomenon "differential reactivity". The same condition was obtained by Coleman (1) in the root tip chromosomes of *Trillium grandiflorum* Salisb. and in this laboratory, in the microspore chromosomes of *T. erectum* (13, Fig. 17).

In arctic species of *Ranunculus* and grasses, Flovik (5, 6) found an exceptionally high number of secondary constrictions in most cases. In some species, e.g., *R. pygmaeus* Wg., three *Phippsia* species, and *Arctophila fulva* Rupr., so many constrictions were found that the chromosomes had a beaded appearance similar to that observed in *Crepis* and *Secale*. The resolution of these constrictions depends to some degree on the choice of fixatives, those containing little or no acetic acid being most satisfactory.

In brief, after appropriate fixation "compound chromomeres" and/or secondary constrictions have been seen in the somatic chromosomes of *Allium*, *Crepis*, *Secale*, *Ranunculus*, and several genera of grasses. After cold treatment secondary constrictions and differential segments have been found in the somatic chromosomes of *Crepis*, *Paris*, *Fritillaria*, and *Trillium*; in *Triticum*, *Trillium smallii*, and *T. Tschonskii* secondary constrictions have also been obtained after pretreatment of the material with chloral hydrate. These conditions may all be considered as "differential reactivity" and, although proof is not yet available, it seems probable that they are all manifestations of the same phenomenon. As both mitotic and meiotic chromosomes are now known to have their chromonemata coiled at metaphase and anaphase, it may be concluded that the "chromomeric" appearance at these stages bears no more than a superficial resemblance to the chromomeric structure of early prophase.

Since such differential reactivity almost certainly indicates a basic differential pattern in the chromosomes, knowledge concerning the origin and behaviour of such regions may be of considerable importance in the elucidation of chromosome structure, behaviour, and constitution. There are many angles from which the study of differential reactivity may be investigated, eventually involving chemical methods, but it is the opinion of the authors that before such methods are employed an exhaustive study of a purely cytological nature should be made. The experiments to be described in this paper represent their initial approach to this problem. Although they are necessarily preliminary in nature and cannot justifiably be used as a basis

for extensive speculations, they have brought to light several facts which, we believe, will eventually prove of considerable importance in the final elucidation of this problem.

Materials and Methods

Most of the present investigation has been based on *Trillium erectum* but some studies have also been made on *T. grandiflorum*, *T. undulatum* Willd., and *Secale cereale* var. Horton and Rosen. Root tip mitoses were studied in all cases. Cold treatment was provided by a commercial electric refrigerator that maintained a fairly constant temperature of 3° C. Root tips of all *Trillium* species were fixed in three parts absolute alcohol and one part glacial acetic acid, macerated in 45% acetic acid at 60° C., and stained with aceto-carmine. This method was also used on *Secale* but better results were obtained by fixing in La Cour's 2BD with subsequent staining by the Feulgen technique.

Observations were made with a Zeiss 1.5 mm., 1.3 N. A. objective and 7× oculars. A Zeiss 3 mm., 1.4 N. A. objective and a 7× ocular were used when photomicrographs were taken.

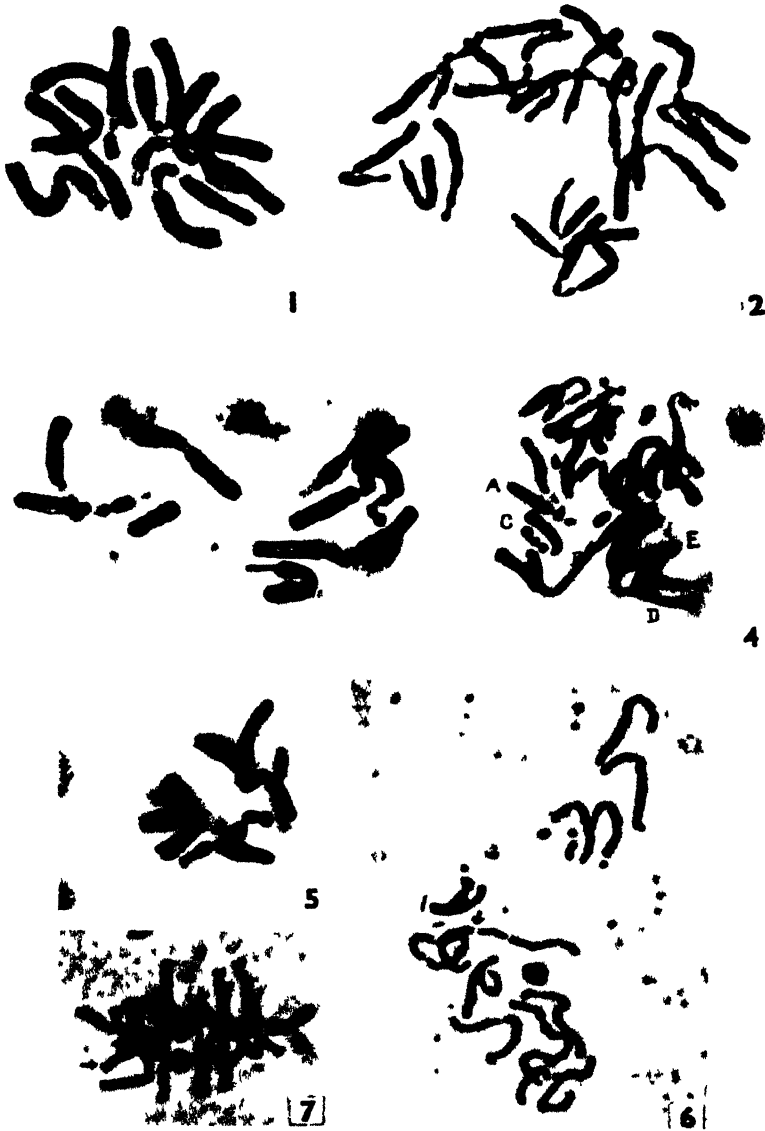
Observations

THE EFFECT OF COLD TREATMENT ON THE SOMATIC CHROMOSOMES OF RYE

Grains of rye were germinated in sawdust and subjected to a temperature of 3° C. for 72 hr. Late prophase chromosomes were found to be beaded in appearance as described by Shmargon (12). Metaphase and anaphase chromosomes were similarly affected, but not to the same extent. The appearance of the affected regions at these stages was similar to that of the differential segments in *Trillium* (Figs. 6 and 7).

THE EFFECT OF COLD TREATMENT ON THE SOMATIC CHROMOSOMES OF *Trillium*

As described by Darlington and La Cour (3) exposure to cold for a sufficient length of time prevents certain regions of the metaphase and anaphase chromosomes of *Trillium* from staining normally. As a rule these regions are understained and are only about one-half the diameter of the unaffected portions of the chromosome (Figs. 1 to 5). Occasionally, however, these regions have been found to be understained but not noticeably narrower. Also, on occasion, regions much narrower than normal appear to be well stained. These conditions are, however, rare. The terminally situated differential regions in *Trillium* are usually quite long whereas interstitial ones are ordinarily very short. For the most part the interstitial segments are within the proximal third of the chromosome arm but several chromosomes, notably the *D* chromosome of *T. erectum*, *A* of *T. grandiflorum*, and *C* of *T. undulatum* (Fig. 4) characteristically have differential segments in the distal third of one arm.



EXPLANATION OF FIGURES

FIGS 1 2 3 Root tip chromosomes of *Trillium erectum* L. showing differential segments
FIGS 1 and 3 are metaphase Fig 2 anaphase ca 700×

FIG 4 An anaphase cell from a root tip of *Trillium undulatum* Willd. Chromosomes A B, and C show differential segments while D and E appear to be normal ca 700×

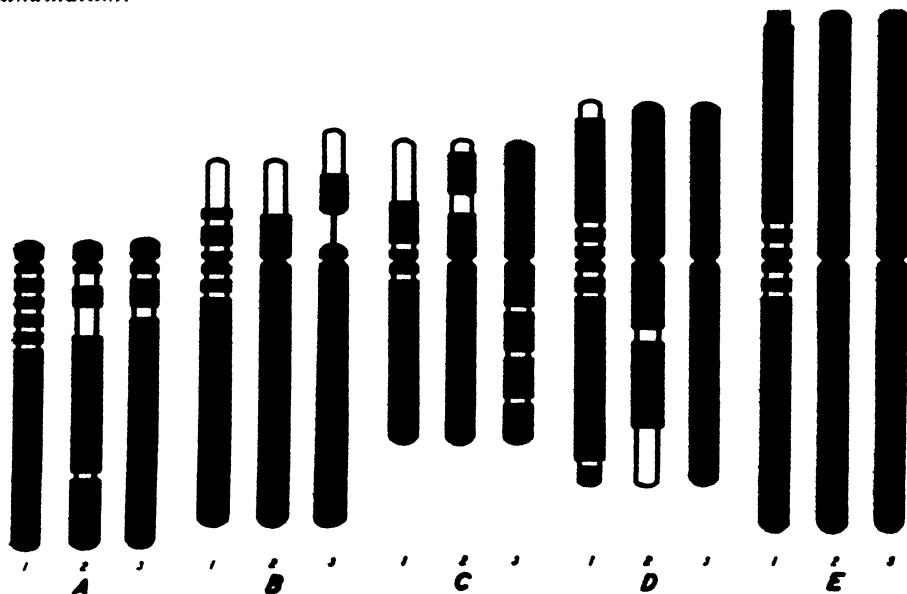
FIG 5 A metaphase cell from a root tip of *Trillium grandiflorum* Sibth showing differential segments in the B, C, and D chromosomes Note that chromosome E is normal in appearance ca 700×

FIG 6 Early metaphase from a root tip of *Secale cereale* L var Rosen showing an interstitial differential segment ca 1100×

FIG 7 A metaphase plate from a root tip of *Secale cereale* L var Horton Note terminal differential segment similar to those found in *Trillium* ca 1100×

The Specificity of the Differential Segments

Although the size of the differential segments is subject to some variation, they are highly specific as to position. Each chromosome has a characteristic pattern. The diagram in Text-fig. 1 shows the position and average size of all regions, in all three species, that have at one time or another shown the reaction. These diagrams represent all the regions found in the present experiments. Chromosomes *A* and *C* of *T. erectum*, *A*, *B*, *C*, and *D* of *T. grandiflorum*, and *A*, *B*, and *C* of *T. undulatum* have at times been found to be affected in all susceptible regions. No differential segments were found in the *E* chromosome of *T. grandiflorum* or in the *D* and *E* of *T. undulatum*.



TEXT-FIG. 1. A diagram showing the size and position of all differential regions that have been observed in the somatic chromosomes of *Trillium*. (1) *T. erectum* L., (2) *T. grandiflorum* Salisb., and (3) *T. undulatum*, Willd. Cross-hatched regions appear only rarely.

Some regions are apparently more readily affected than others. This is indicated in Table I where seven plants of *T. erectum* in which 90% or more of the chromosomes are affected have been classified to show their distribution in the various possible combinations of terminal, terminal plus interstitial, and interstitial regions. It is quite obvious from these data that the terminal regions of chromosomes *B*, *C*, and *D* are more easily affected than their interstitial segments. Part of this difference may be due to our inability to see segments that are not markedly affected, but it is unlikely that the disproportion is entirely due to this factor, since there seems to be little difficulty in discerning the subterminal region in the *D* chromosome which as a rule is less obvious than other interstitials. It has not been possible to differentiate accurately among the proximal differential segments and, therefore, not possible

TABLE I
THE DISTRIBUTION OF DIFFERENTIAL SEGMENTS IN *Trillium erectum* L.

Segments	Chromosome	Material										Subtotal	72-R-336	72-R-676	Total
		72-D ₁ -96	72-F ₁ -96	72-M-96	72-O-90	72-P-96	72-Q-96	72-R-96							
Normal	A	4	2	2	6	12	0	0	0	26	1	1	28		
	B	1	6	0	6	1	0	0	0	14	0	1	15		
	C	1	3	0	2	3	0	2	2	11	0	2	13		
	D	4	2	9	4	9	4	0	0	32	0	0	32		
	E	0	5	1	1	3	0	0	0	10	0	0	10		
1 Terminal	A	0	0	0	0	0	0	0	0	0	0	0	0		
	B	15	23	33	3	32	9	7	122	15	4	141			
	C	26	39	45	35	96	22	18	281	31	13	326			
	D	7	21	22	9	27	8	4	98	5	3	106			
	E	0	0	0	1	0	0	0	1	0	0	1			
1 Terminal + 1 interstitial	A	0	0	0	0	0	0	0	0	0	0	0	0		
	B	10	14	13	9	51	10	10	117	9	14	140			
	C	1	0	0	3	2	0	1	7	3	3	13			
	D	9	19	11	8	29	7	7	90	15	13	118			
	E	0	1	0	0	0	0	0	1	0	0	1			
1 Terminal + 2 interstitial	A	0	0	0	0	0	0	0	0	0	0	0	0		
	B	2	3	1	7	7	2	3	25	6	3	34			
	C	0	0	0	2	0	0	0	2	0	0	2			
	D	2	2	1	9	9	0	5	28	10	5	43			
	E	0	0	1	1	0	0	0	2	0	0	2			
1 Terminal + 3 interstitial	A	0	0	0	0	0	0	0	0	0	0	0	0		
	B	0	0	1	1	1	0	0	3	0	0	3	3		
	C	0	0	0	0	0	0	0	0	0	0	0	0		
	D	0	2	0	1	2	0	1	6	2	1	9	9		
	E	0	0	0	0	0	0	0	0	0	0	0	0		
1 Interstitial	A	13	23	28	6	33	6	2	111	3	6	120			
	B	0	4	0	8	2	1	0	15	0	0	15			
	C	0	0	0	1	0	0	0	1	0	1	2			
	D	4	3	5	10	13	2	1	38	2	1	41			
	E	0	4	8	4	24	4	3	47	2	3	52			

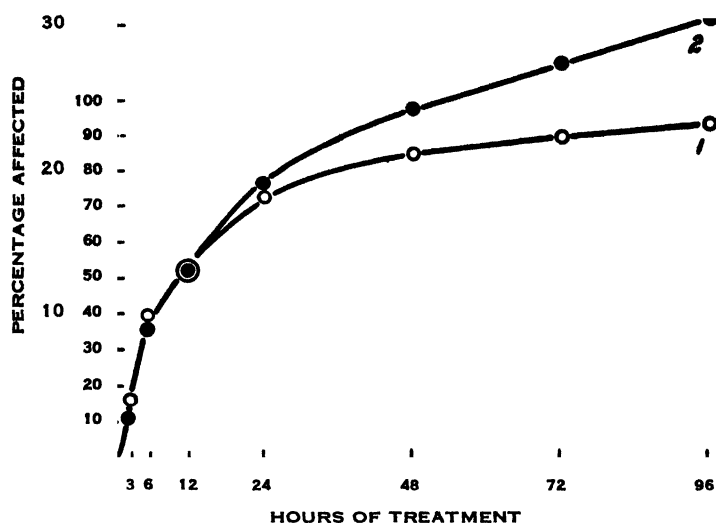
to say whether or not these differ from each other in the ease with which they may be affected. A classification as to the ease with which segments may become differentially reactive is not without exceptions, chromosomes having sometimes been found in which only the ordinarily less susceptible regions were affected.

Regions, ordinarily unaffected, lying between those that are usually susceptible may show differential reactivity on occasion. In the *A* chromosome of *T. erectum*, for instance, all five proximal segments may become merged to form one differential segment involving about a third of the long arm. Usually in such a case the reaction is less marked in the intervening portions than in the characteristically differential segments. Similarly, in the *E* chromosome of *T. erectum* the whole attachment region between the "normal" differential segments may lose its staining capacity; likewise the terminal segment of the *B* chromosome and the adjacent interstitial segment may merge, often reducing the intervening region to the point where it is morphologically indistinguishable from them. These intersegmental regions may belong to the less susceptible category of differential segments and, therefore, be capable of being affected regardless of the neighbouring regions or they may be secondarily affected by extension of regions already affected. However, since they are usually less markedly affected, the latter interpretation is favoured. It is also possible then that different sizes of both terminal and interstitial regions are due to invasion of normally unaffected areas. This is particularly probable in the interstitials of *T. grandiflorum* where the segments may involve from one to five gyres of the somatic coil and where the diameter and pitch are apparently without much variation. There is no evidence suggesting that stretching of the regions has taken place.

The Rate of Differentiation

The present investigation has been particularly concentrated on obtaining some knowledge of the rate with which differential segments appear. For this purpose plants of *T. erectum* were exposed to a temperature of 3° C. for times ranging from 15 min. to four weeks. Differential regions first appear after 30 min., and by 96 hr. nearly all chromosomes are affected. Owing to the small number of suitable root tips on the rhizome it was necessary to construct the general curve from a number of overlapping partial curves. Differences between plants are relatively small and may, for the present, be ignored.

Of several ways in which the relationship between duration of treatment and the degree of effect may be determined only two are suitable at present. These are (1) plotting the percentage of chromosomes affected against the duration of treatment and (2) using the regions indicated in Text-fig. 1 as a standard, and plotting the percentage of those obtained against the duration of treatment (Table II). In Text-fig. 2 both curves have been plotted.



TEXT-FIG 2. Curves showing the rate of differentiation. Curve 1 was obtained by plotting the percentage of chromosomes affected (figures in small type) against duration of treatment and Curve 2 by plotting the percentage of "standard" regions obtained (figures in large type) against duration of treatment

These curves cover only the first 96 hr. Owing chiefly to the deleterious effect of long exposure to cold on the root tips only this part of the curve has been investigated in detail.

Curve 1 represents the rate with which the chromosomes become differentially reactive while Curve 2 indicates not only the rate with which the chromosomes are becoming differentially reactive but also the degree to which they are affected. Curve 1 will, of course, reach a plateau when all the chromosomes are affected and this condition is virtually attained after 96-hr treatment. Curve 2 is limited only by the maximum number of regions that the chromosomes may have, but in so far as present observations go there is little rise in it after 96 hr, when only about 30% of the "standard" regions are affected. This does not necessarily mean that a maximum has been reached but may mean only that the treatment is inadequate. It is also possible that any degree of effect beyond a certain threshold is cell-lethal and this threshold would then be the maximum. The two curves are similar for the first 24 hr and they then diverge, indicating not only that more chromosomes are becoming differentially reactive but also that the degree to which the chromosomes are affected is increasing. While the curves have not been investigated beyond 96 hr. in sufficient detail to warrant very definite conclusions it is quite obvious that the increase in degree of effect beyond that time is very slight (Table II).

These curves are, of course, compounds of those for the five chromosomes. At this stage of the investigation the authors do not feel that the amount of data available is sufficient to warrant detailed discussion of the individual

chromosomes. The percentage of "standard" regions affected after various exposures for each of the five chromosomes has, however, been determined (Table IIIa). From the results tabulated (Table IIIb) it seems that there are quite marked differences between the chromosomes as to the rate with which they show differential segments.

Recovery

Of equal importance to the rate of appearance of the differential segments is the rate with which they disappear on cessation of treatment, but lack of suitable material has made it impossible so far to determine recovery rates in adequate detail. From such experiments as have been performed, however, one definite fact has been determined; namely, that after a 90-hr. exposure to cold the chromosomes return to normal in about 10 hr. at room temperature, most of the recovery occurring within four or five hours. This rapid recovery relative to the rate at which the chromosomes become affected is not unexpected since the rate of mitosis must also be increased at room temperature.

Heterogeneous Chromosome Pairs

Darlington and La Cour (3) have illustrated a number of homologous pairs of chromosomes that differ in number, position, or size of the differential segments or in various combinations of these. They have designated such pairs as "hybrid." The present authors do not feel that this term is justified since it implies genetic hybridity, and prefer, pending further investigation, to call them heterogeneous chromosome pairs.

For the purposes of discussion such pairs have been divided into three classes: (1) pairs in which only one member contains differential segments, (2) pairs differing in the number of these regions, and (3) pairs differing in the length of the terminal regions.

From the fact that increased time of treatment results in a higher percentage of the chromosomes being affected, it seems obvious that Class 1 will eventually be eliminated. Therefore, pairs in which only one member is affected need not be considered further. Within the range of times studied the frequency of heterogeneous pairs of Types 2 and 3 remains relatively constant. These may, therefore, be considered as possible structurally and genetically different or "hybrid" pairs but there are several facts that indicate that such an assumption is not entirely justified. In the first place until the degree of differential reactivity reaches a point of stability a heterogeneous pair must be considered as being potentially similar. In the second place, even if a point of apparent stability is reached (as it seems to be after 96-hr. treatment), it is not known that this stability continues indefinitely. In the third place, even if it does, it may not represent the highest degree to which the chromosomes are capable of being affected; under different treatment it may be higher. Fourthly, the distribution of the affected regions may be subject to chance variation. In the fifth place, heterogeneous pairs do not necessarily indicate genetic hybridity within a plant since a chromosome may fall into

more than three categories regarding the number and distribution of its differential segments.

Variability

Differential segments were found to vary greatly in length, this variability persisting throughout the range of treatments studied. Terminal regions were found to be more variable in *T. erectum* and interstitial regions in *T. grandiflorum*. The cause of this variability is unknown. It seems unlikely that it is due to stretching since long regions are not usually narrower than short ones nor is the pitch of the somatic coil noticeably different.

Darlington and La Cour (3) found similar variability in the length of differential segments. They have explained this variability in terminal segments as being the result of "sticking" and failure of normal separation at anaphase. Breaking of the bridge produced by sticking in these regions may result in a duplication in one daughter chromosome and a deficiency in the other. It is difficult, however, to see how such a mechanism could produce variations in the lengths of interstitial regions. In *T. grandiflorum* and *T. stylosum* Nutt. Darlington and La Cour found that 58 out of 127 anaphase figures showed faulty separation of one or more chromosomes. The *T. grandiflorum* material used in the present study has not been examined sufficiently to determine the frequency of faulty separation but out of 13 clear and undamaged anaphase cells only one showed "sticking". Similarly, in 65 cells of *T. erectum* only one case of "sticking" has been found; this was in a cell in which the chromosomes showed no differential segments. It seems unlikely, therefore, that sticking occurs with a high enough frequency to explain the length differences found in terminal regions in our experiments. A more probable explanation of this variation in length appears to us to be extension of the effect to parts of the chromosome adjacent to the differential segment.

Discussion

Darlington and La Cour's suggestion that this phenomenon probably involves the nucleic acid metabolism of the chromosomes is a plausible one, but until more is known concerning nucleic acids in the cell this relationship is purely speculative. They state that the differential segments represent the heterochromatic regions of the chromosomes and identify these with the "chromocentres" of the resting nucleus. While such a relationship may exist our preliminary investigations indicate that far more intensive study must be made before this can be considered as more than an interesting speculation.

Within the differential regions the major effect appears to be in the matrix in that its staining capacity is more reduced than that of the chromonema. The specificity as to position, however, shows that the phenomenon is under the control of the chromonema or the gene-string.

The authors cannot agree with Darlington and La Cour that differences in size, number, or position of differential segments between homologous chromo-

somes is proof of hybridity whether comparisons are made within or between plants, since heterogeneous chromosome pairs may be equally well considered as potentially similar pairs.

Any interpretation of the shape of the reaction curves presented must await the elucidation of factors that are at present unknown. One of these is probably the rate of mitosis under different temperature conditions.

It appears that not all potentially differential segments are equally susceptible to cold, since certain ones become differentiated far more frequently than others, whereas some occur very rarely even though they appear in a number of different plants. The order in which the regions become affected is not, however, always the same for in rare cases the chromosomes may show one of the least susceptible regions without also having the more susceptible ones.

The fact that the *Trillium* species investigated have different differential patterns suggests that this phenomenon may be of unique value in cyto-systematic studies. Such studies are now being made of the genus *Trillium* in this department as a concurrent problem in collaboration with Dr. Pierre Dansereau of the Montreal Botanic Gardens.

In addition to its own obvious utility and interest the existence of differential reactivity is of far wider import in that it is further indication that the chromonema is not uniform throughout its length but is differentiated longitudinally as to chemical structure and behaviour. An explanation of the phenomenon of differential reactivity will, therefore, probably involve a solution to some of the problems of submicroscopic structure. It is the authors' opinion that the eventual solution will be found only by combining the results of experiments undertaken from several points of view, i.e., physical, chemical, and biological.

Acknowledgments

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THE EXPERIMENTAL INDUCTION OF PARTHENO-CARPIC STRAWBERRIES¹

By A. W. S. HUNTER²

Abstract

The parthenocarpic development of strawberry fruits was induced by spraying unpollinated blossoms with solutions of indolylbutyric acid, 1-naphthylacetic acid, and colchicine, and by dusting the blossoms with powdered acenaphthene. Fruits also developed from blossoms that had not been directly treated. This is explained on the basis of translocation of the chemical, or some other substance, from treated to untreated blossoms.

It is suggested that colchicine, and possibly the phytohormones, induce parthenocarpy by acting as mobilizers of another substance or substances that move into the ovary and there initiate development.

The experimental production of diploid parthenogenesis in the strawberry as a means of securing homozygosity has engaged the attention of the writer. In an experiment with growth promoting substances and polyploidizing agents the results from the standpoint of parthenogenesis were practically negative, but parthenocarpic fruits were produced in abundance. This account has been prepared since it is believed to add to the knowledge of the methods by which parthenocarpy may be produced.

Methods

During the winter of 1938-1939 plants of three pistillate strawberry varieties, Louise, Portia, and Simcoe, were grown in pots at one end of a greenhouse under conditions such that the chances of accidental pollination were negligible. The blossoms on these plants were treated with the growth promoting substances, indolylbutyric acid and 1-naphthylacetic acid, and with the polyploidizing agents, colchicine and acenaphthene. The indolylbutyric acid, naphthylacetic acid, and colchicine were applied in concentrations of 1.0, 0.5, and 0.25%. A single concentration of one chemical was applied to a separate plant of each variety.

The indolylbutyric and naphthylacetic acids were dissolved in 95% alcohol and the solutions of colchicine were made up in a lanolin emulsion (14). The solutions were applied with an atomizer shortly after the blossom opened. Since acenaphthene is practically insoluble in both water and alcohol, finely powdered crystals were dusted full strength over the stigmas with a camel-hair brush. Treated flowers were tagged and were not sprayed or dusted again unless some of the stigmas had not turned brown after the first application. Since in the majority of cases flowers were deliberately treated once only, the results may be considered as produced by single applications.

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Check treatments were made with 95% alcohol and with lanolin emulsion. Unfortunately, plants of Louise, Portia, or Simcoe were not available for this purpose. Two other pistillate varieties were used instead and will be referred to as Seedling A and Seedling B.

Results and Discussion

Parthenocarpic fruits have been produced in a number of different genera and species by application to the stigmas of pollen extracts and growth promoting substances and related chemicals (2, 4, 5, 6, 8, 13, 16). The only previously reported work of this nature on strawberries, as far as the author knows, is by Gardner and Marth (2) who produced ripe fruits by spraying the blossoms with 0.1 and 0.05% indolylacetic acid.

The number of flowers treated and the number of fruits that matured from these flowers are given in detail in Table I. Several of the plants failed to blossom and consequently the treatments intended for these plants had to be omitted.

TABLE I
BLOSSOMS TREATED AND MATURE FRUITS HARVESTED

Treatment	Variety									
	Louise		Portia		Simcoe		Seedling A		Seedling B	
	Number of flowers treated and fruits developed therefrom									
	Tr.	Dev.	Tr.	Dev.	Tr.	Dev.	Tr.	Dev.	Tr.	Dev.
Indolylbutyric acid										
1 0%	4	2	6	6	6	6				
0 5%	8	8	7	7	3	3				
0 25%	8	8	6	6	3	3				
1-Naphthylacetic acid										
1.0%	3	1	5	1	20	2				
0.5%	3	3	1	1	0	1				
0 25%	5	5	8	7	10	9				
Colchicine										
1 0%	9	0	6	1	33	0				
0 5%	0	—	8	7	0	—				
0 25%	14	11	7	7	4	3				
Acenaphthene (dust)	9	8	7	0	12	3				
Check										
95% alcohol							12	0	6	0
Lanolin emulsion							13	0	4	0

No parthenocarpic fruits were produced by treatment of the blossoms of Seedlings A and B with 95% alcohol or lanolin emulsion alone. The blossoms withered and turned brown after a time in the same way as untreated flowers that had not been pollinated. Genetic differences naturally exist between

these two varieties and Louise, Portia, and Simcoe but it is considered unlikely that they are sufficient to invalidate the use of Seedlings *A* and *B* as the checks. Therefore, the 95% alcohol and the lanolin emulsion are believed to have had no part in the production of parthenocarpic fruits.

Parthenocarpic fruits were produced in abundance by all concentrations of indolylbutyric and by the 0.5 and 0.25% concentrations of naphthylacetic acid and colchicine. The 1% naphthylacetic acid initiated fruit development but it also caused considerable damage to the leaves and pedicels. The pedicel injury was so severe that most of the fruits did not reach maturity. The 1% colchicine caused no damage to the plants but only one mature fruit (on Portia) was produced. This was small and misshapen. Under the conditions of their experiment, Gardner and Marth found never more than one fruit on an inflorescence developing to maturity. No such limitation occurred in the present experiment, several well developed fruits being borne on many of the inflorescences following treatments with indolylbutyric acid, naphthylacetic acid, and colchicine. Acenaphthene was not as effective as the other substances in inducing parthenocarpy, although almost all the flowers treated showed some initial fruit development and many of the fruits grew to a length of approximately one centimetre. In all three varieties the mature parthenocarpic fruits were average in size and normal in appearance (Fig. 1) but the achenes were smaller than those on pollinated fruits.

In addition to the fruits that developed from treated blossoms it was also noticed that, on inflorescences bearing flowers treated with indolylbutyric acid, naphthylacetic acid, and colchicine, several flowers that had not been tagged as having been treated showed some carpel enlargement and eventually mature fruits developed. This might have been due to the accidental spraying of unnoticed open flowers. In order to be certain that this was not the case, 53 unopened flowers were tagged at the completion of the spraying programme. In 30 of these flowers there was no development at all but from 12, distributed at random over the different varieties and treatments, ripe fruits developed and in 11 there was initial growth but fruits did not mature. Therefore it is certain that this development was not due to the accidental spraying of open flowers. Since no particular care was taken to prevent the sprayed solutions from reaching parts of the plants other than the blossoms, the stimulation that caused these fruits to develop may have been produced in either of two ways:

1. The presence of the chemical on the outside of the folded sepals may have been sufficient to induce the development of fruits.

2. The chemical or some other substance which it mobilized may have been translocated from the treated to the untreated flowers. The second assumption appears to be the more logical and is supported by a later experiment in which a shield of paper was placed around each blossom as it was being sprayed. The solutions reached no part of the plant except the treated flowers, notwithstanding which several untreated blossoms developed into mature fruits as in the original experiment.



FIG. 1. A—*Simcoe*. Blossoms sprayed with 0.5% indolylbutyric acid.
B—*Simcoe*. Blossoms sprayed with 0.25% naphthylacetic acid. All the fruits are on two inflorescences.
C—*Portia*. Blossoms sprayed with 0.5% colchicine. The size of the fruits is a characteristic of the variety.

These fruits which matured were normal in appearance but the achenes were small and underdeveloped as they were in the fruits produced by direct treatment of the blossoms. In the hand pollination of strawberries in the greenhouse the writer has never observed the development of unpollinated blossoms. Presumably the development of untreated flowers is associated with an excess of the stimulating substance in the treated ones. The amount of stimulant produced in pollinated flowers is probably insufficient to affect those not pollinated.

Growth responses other than parthenocarpy were also produced by indolylbutyric acid and to some extent by naphthylacetic acid. With both these substances there was a marked elongation and twisting of the pedicels. Gardner and Marth (2) reported a similar occurrence in strawberry plants sprayed with 0.1% indolylacetic acid. With indolylbutyric acid another effect was the formation of adventitious roots on the scapes in the region of the first node and also at the bases of both scapes and petioles. Bending of the leaves and stems and adventitious root formation have been induced in several plant varieties by local applications of synthetic hormones (10) and by applications to the soil in which the plants were growing (11). In the present experiment no care was taken to protect the foliage or the soil when the blossoms were being sprayed, so that appreciable amounts of the chemical must have been received by the soil and by the leaves and other parts of the plant. This could account for the above results.

The fact that parthenocarpic fruit development in the strawberry may be induced by treatment with colchicine deserves further consideration. Indolylbutyric and naphthylacetic acids both possess growth promoting properties. While little study has been given to colchicine in this respect, not much evidence of growth promoting activity has been found (1, 3), although Havas (9) reported adventitious root formation on the stems of *Impatiens balsamina* following an application of a 2.5% paste of colchicine in lanolin. In the present experiment the plants treated with colchicine exhibited no evidence of epinastic or other growth responses such as were observed in the case of those treated with indolylbutyric acid and naphthylacetic acid. Similar results were also observed in an earlier experiment performed by the writer, wherein various concentrations of indolylbutyric acid, naphthylacetic acid, and colchicine were injected into the trunks of plum trees.

However, more definite information was required on the possible phytohormonal properties of colchicine. In order to obtain such information a series of experiments was conducted with colchicine and indolylacetic acid. Indolylacetic acid was chosen because of its frequent use in phytohormonal studies of this nature. Colchicine and indolylacetic acid were compared for their effect on the curvature of the split stems of etiolated seedlings of *Pisum sativum* (13), the inhibition of lateral bud growth on decapitated tomato plants (10), the production of epinastic responses by tomato plants following applications to the plant (10) and to the soil in which the plant was growing (11). In these experiments the only indication that colchicine has phyto-

hormonal properties was the formation of adventitious root primordia on the stems of tomato plants following local applications in lanolin. Root primordia were produced by the same concentrations of indolylacetic acid but none were found on the check plants. Thus little if any evidence is added to the data already available which would indicate that colchicine possesses to any appreciable extent those properties commonly associated with plant growth substances.

However, since colchicine and the growth promoting substances both initiate parthenocarpy they must have properties in common. It is possible that the induction of parthenocarpy by the growth promoting substances is not altogether due to the fact that they are growth substances, but that it is the effect of one or more of these common properties. Gustafson (7) pointed out that the plant hormones should not necessarily be considered the only factor involved in the initiation of parthenocarpic fruit growth, and suggested, by analogy with Went's (15) terminology, the term "carpocaline" for a substance or substances that, under the influence of auxin, move into the ovary of a flower and there cause growth to take place. According to Went's interpretation, the naturally-occurring auxin in the plant acts as a mobilizer. It would seem possible therefore, that it is this mobilization property which is common to colchicine and the growth substances and which is responsible for the induction of parthenocarpic fruit.

The "seeds" from all the parthenocarpic fruits were sown as soon as the fruits were ripe. One seedling grew from a fruit that developed on the *Portia* plant treated with 0.25% colchicine. Unfortunately this plant died while still in the seedling stage. The death of the plant was due to an accident and not to any lack of viability. However, root tips had been obtained from which it was determined that this seedling, like *Portia*, was an octoploid ($2n = 56$). This plant may have been parthenogenetic or it may have been the result of accidental pollination. From the fact that only one plant was secured from so many "seeds", one would suspect the latter, but a selfed population would have been necessary in order to decide this point.

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SEED DEVELOPMENT IN *MEDICAGO* (ALFALFA) HYBRIDS

I. THE NORMAL OVULE¹

BY HELEN M. FARLEY² AND A. H. HUTCHINSON³

Abstract

Seed production in *Medicago* (alfalfa) hybrids is related to many factors, primarily the cytological disturbances due to hybridization. The establishment of new balances results in strains which may occupy different habitats. Deficiencies may appear at one or more of the many stages during the complete process of growth and development. This account on the normal ovule, as a standard for comparison, deals particularly with the histogenesis and development of the nucellus, gynosporcs, integuments, funiculus, female gametophyte, embryo and endosperm. Some of the food and nutrition problems associated with seed production are considered. A series of papers dealing with special phases of seed development in hybrid *Medicago* is in progress.

Introduction

Cultivated forms of alfalfa are notably low seed producers. Previous descriptions of the histological development of the ovule and seed in alfalfa have applied to plants designated as *Medicago sativa* L., presumably a pure species; since the plants described in this paper and most of the northern, commercial forms of alfalfa are of hybrid origin, it has seemed to the authors to be significant to determine the degree of variation from the "original species" as well as the variation within a hybrid group.

Previous descriptions of ovule development in seed plants have given much emphasis to the direct precursors of the egg and embryo, that is to the female gametophyte, and minimal attention has pertained to the adjacent protective and nutritive tissues. Recent investigation, however, has emphasized the importance of the nutrition balance after embryo formation. The dependence of egg production in plants upon favourable nutritive and protective structures has been taken for granted and the uniform accomplishment of this teleological necessity in development has been assumed, to a high degree. This assumption seems quite contrary to fact, at least in the hybrids of alfalfa examined in the present study. The greater part of this paper deals with the origin and development of nutritive and protective tissues leading to the production of normal eggs, as essential features of seed formation, especially in alfalfa hybrids grown at the University of British Columbia.

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Material

Early History of Medicago

The earliest history of man records the fodder plant, now known as *Medicago*, in Media; probably the species was similar to *M. sativa* L. of the present and these plants were the ancestors of the several, recent, cultivated species and numerous varieties which are widespread, and of great economic importance. Later, it was grown in Persia and was carried to Greece by Alexander the Great; then, to Italy. The Moors are credited with its introduction to Spain from Africa, during the thirteenth century; they called it "alfalfa" or "best of fodder". During the sixteenth century the Spanish introduced it into Mexico and later to South America; from Chile the migrants of the gold rush carried the seeds to California. "By 1850 it had spread eastward to Utah, Colorado, Kansas, Nebraska, Iowa, Illinois, and Ohio" (25). This chain of translocations did not materially change the climatic habitat from its original "Mediterranean" type and the species would appear to have retained the primary characteristics of the ancestor from Media (14).

From Spain "alfalfa" was carried to southern France, and to Geneva during the sixteenth century (14). It seems probable that the term "lucerne" was applied first either to the form (known as "laouzerdo") grown in Southern France (17) or to a Swiss variant of *M. sativa* that was tolerant to mid-temperate conditions (14). German lowland lucerne was first (1710) developed by Cistercian monks in Franconia; later (1730) the evidence indicates that an upland form, sometimes called "*M. media* (Pers.)", and commonly called "lucerne" was selected from *hybrids** of the Franconian *M. sativa* L. \times *M. falcata* (L.). The latter ancestor is believed to have originated in the Caucasus region, an area that is very rich in natural species of *Medicago* at present (11, 24).

"The first recorded attempt to grow alfalfa in the United States of America was made in Georgia in 1736 (25). These early attempts to grow the crop in the East were probably on acid soils which in part explains the unsatisfactory results" (Westover, personal correspondence, 1941). Although occasional fields of "lucerne" were grown on "limestone soil" as early as 1794 (23), the first *Medicago* to be more widely successful in the northern United States and in Canada was derived by natural selection from offspring of the German hybrid "*M. media*" and was cultivated on a Minnesota farm by Wendelin Grimm after whom it was named "Grimm". A similar form was selected in Canada from hybrid plants grown from seed of German origin, and was designated "Ontario Variegated". *M. sativa* is purple-flowered, *M. falcata* yellow-flowered and the hybrids vary in this as well as in other respects.

Origin of British Columbia Hybrids

The British Columbia hybrids are the result of a back-cross between plants of "*M. media*", variety "Ontario Variegated", and a single plant of *M. falcata*

*The problem arising from the difference between a Mendelian hybrid and a hybrid as defined by the International Rules of Botanical nomenclature is recognized by using italics where the word hybrid is used in a taxonomic sense.

var. Don, obtained by Dr. L. S. Klinck from Prof. N. E. Hansen of South Dakota, who formerly imported this variety from Russia. The crosses, made by P. Boving, resulted in six back-cross hybrid plants with marked variation in gene combinations, notably in flower colour, pod shape and size, degree of seed production, extent of rhizome formation, and erectness of habit. Plants have been selected by G. G. Moe, from three generations of hybrids, with particular reference to the economic features: extent of rhizome production, erectness, and seed production; the improvement of these "hybrids" is progressing.

If the reported ancestry is authentic the '*falcata*' lineage was twice introduced in the production of the British Columbia hybrids, once at the origin of the upland German '*media*' and again (1916) from the 'Don' used in the British Columbia experiments. Through '*media*' the remote ancestry '*sativa*' enters, and it is possible that the development of 'Ontario Variegated' may have added another '*sativa*' factor to the sum total of this hybridity.

In this account, since the original British Columbia plants were of multi-hybrid origin they will be designated as *M. media* (B.C.) and *M. falcata* (B.C.), respectively, and their hybrids as *M. media-falcata* (B.C.) hybrids, Generations 1, 2, and 3, respectively.

Methods

Various killing and fixing reagents were used, especially Flemming's, La Cour's, Navaschin's, Bouin's, and Carnoy's. The most satisfactory results were obtained with combinations of alcohol, acetic acid, formalin, and chloroform. Tissues were hardened by osmic and chromic acids and by mercuric chloride.

No one strain was entirely reliable for all tissues. Iron alum haematoxylin (with light green counter stain), safranin with light green, Feulgen's carmine stain, and Semmens' and Bhaduri's light green counterstain were all utilized to advantage for particular stages or tissues.

Records are available for the immediate ancestry of each plant from which flowers were collected, and for the commercial characteristics. Flowers were marked at the time of tripping and subsequent collections were recorded from this reference point. Stages before tripping were estimated, with the same reference point.

The Problem of Determining Normality of Seed Development in Hybrid *Medicago*

Medicago has, notably, a low seed production. Because of the multiplicity of species, strains, and hybrids growing under varying conditions of location and season the factors that contribute toward the non-development of ovules are necessarily many and varied in their relative values (5, 6). The following factors have been suggested. 1. The pollen may be non-viable. 2. If self-pollination occurs self-incompatibility may intervene (1, 9, 13). 3. The usual insect pollination is dependent upon the variable activity of bees under different conditions (19). 4. The rate of pollen tube growth may not parallel the

normal development of the ovule and fertilization may not be consummated (4, 12). 5. The food supply to the pollen tubes, to the integuments, to the nucellus, to the gametophytes, to the endosperm, and to the embryo may not be properly apportioned or timed, and normal development may not continue. 6. Finally, parasites may destroy the seed at some stage of development (21). Any deficiency in one of these chief events in the processes involved in seed production may be sufficient to inhibit the normal production of viable seed. The problem in any particular case is to determine the limiting factor or cumulative group of factors. In order to determine deficiencies a standard of the "normal" is required. Although many have noted the variants in *Medicago*, the complete series of stages in all parts of the ovule that are essential for the final development of viable seed has not been entirely evaluated. *The normality of development in Medicago is not to be judged on a basis of the greatest number of cases following a particular course, but preferably upon the harmonious succession and co-ordination of stages that attain the goal of seed production.* It is proposed to deal in later papers with the development of androspores and of the male gametophyte, polyploidy, and aneuploidy in relation to autosynthesis and compatibility, and the factors that lead to normality or to deficiencies in the processes of seed development.

The complex genesis of plants of "*M. falcata*" and "*M. media*" used as the progenitors of the British Columbia hybrids, added to the hybridization of the experimental B.C. *Medicago* lines, has resulted in a complex of variants, partly as a result of the Mendelian assortment and recombination of genotypic characters and partly as induced disturbances of the chromosome complements and their multilateral associations. These involve the physiological and genetic interrelations of the co-vivial generations included in the seed: the haploid gametophytes, male and female, the diploid sporangium, and the triploid endosperm. When polyploidy (20) ensues the balance is in further jeopardy. If new, successful, true-breeding strains are to be obtained, there must be established new equilibria that satisfy the requirements of growth vigour, of adjustment toward the particular environment, of intraracial compatibility, and of inter-racial incompatibility.

In the maze of variability, the recognition of a standard of normal development is the first problem of the geneticist; this standard may vary within certain limits of known divergence, limits beyond which deleterious results occur and are expressed as a marginal lowering of seed production or of seed viability.

Early Development of the Ovule, and Gynosporogenesis*

The single carpel of the *Medicago* flower develops from a terminal or sub-terminal, crescentic primordium on the floral axis and rapidly produces a distally open hemicylinder, with a suture along the infolded margins of the wall. Later, the margins fuse and the upper portion becomes constricted

*The terms "gynospore" and "androspore", as suggested by J. Doyle and by L. W. Sharp and as accepted by Thomson (22, p. 43), have been used in this account.

to form the style; the somewhat inflated lower portion, the ovary, produces ovules near the margins of the infolded wall. When the elementary placenta is four cells in thickness the first differentiation of ovular meristem occurs; the dermatogen (outer tunica) actively divides anticlinally, extending the surface, and the meristematic margins of the ovary wall are thickened by coincident periclinal divisions of the hypodermis. On the inner side of the marginal fold the hypodermal layer (outer periblem or inner tunica) divides by regular periclinal divisions; on the outer side of the fold, at intervals along the margin, the ovular mounds develop. These discontinuous extrusions are the result of repeated periclinal divisions of subtending hypodermal cell groups, accompanied by a repression of divisions in adjacent cells (Fig. 3). Ordinarily a group of 10 or more hypodermal cells divide, that is three or four in sectional view, whereas occasionally a smaller number and possibly a single cell may be meristematically distinguished from the adjacent inactive hypodermal cells. This active cell, or cells, may be designated the potential *gynosporangial initial(s)*.

The carpel wall in the placental region is now five cells in thickness except at the ovular primordia which have a sixth and soon a seventh layer; the *primary hypodermal cells* have divided periclinally to produce *secondary hypodermal cells* and inner cells, which may be known as *basal* (tapetal) or *endoparietal* cells. Normally, a single secondary hypodermal cell, centrally located within a group, becomes larger, and denser, and differentiates into an *archesporial cell*. This, in turn, divides periclinally to produce the *gynosporocyte* and an *exoparietal cell*. Concurrently or shortly thereafter the endoparietal (basal) cell divides periclinally, giving an axial row consisting of four cells, a gynosporeocyte, one exoparietal, and two endoparietal cells (Fig. 4). Not infrequently several rows develop similarly and several gynosporeocytes are formed (Fig. 6). These may produce four gynospores each. Normally, when a single axial gynosporeocyte differentiates, the adjacent, somewhat distorted rows of similar hypodermal origin, develop as nucellar tissue, lateral to the axial row (Figs. 3 to 8). Normal development here, as in the case of the earlier primordia, is the result of the co-ordinated activation of certain cells and the partial inhibition of division in others; the former become spore producing, the latter are protective.

In ovules in which a single primary hypodermal cell is present the axial row is contiguous to the epidermis, laterally. Where several secondary hypodermal cells produce gynosporeocytes some of the latter soon disintegrate, resulting in separation from the remaining cells (Fig. 6). The normal development follows neither of these extremes; several primary hypodermal cells divide periclinally each to form the same number of tiers (Fig. 3); one central group only divides and differentiates to produce the axial row; the other tiers give rise to an adjacent several-layered jacket enclosed by the epidermis. As indicated, the several-layered nucellar wall is partly dermal and chiefly hypodermal in origin.

Normally, the gynospore divides meiotically and produces four gynospores which may be linear in arrangement but more frequently the outer cell, resulting from the heterotypic division, divides transaxially or obliquely. Meanwhile the primary exoparietal cell divides periclinally into two, then three exoparietal cells while the endoparietal cells divide in the transaxial and "ipseaxial" planes, successively (Figs. 7, 8, 9). These divisions are rapid and the resulting columella (basal axis) becomes four to eight cells in length and of four, seldom nine, tiers transversely, that is two, seldom three, cells in each axial plane (Figs. 5 to 8, 10, 12).

At this stage there are variations from ovules with an axial row of four gynospores, two parietals, and four endoparietals (columella) covered by a single-layered epidermis to a much more massive ovule, with 16 gynospores (some deficient), 12 exoparietals, a columella of 90 (\pm) endoparietals and a several-layered wall of hypodermal origin, covered by the epidermis. Normally, the axial row consists of four gynospores (three deficient and the inner with abundant stored food), two exoparietals, and 24 endoparietals, constituting a columella of four tiers; the hypodermal wall is three cells in thickness near the base and two near the apex, the outer jacket of epidermal cells is single-layered. The primordia of the two integuments form basal rings, adjacent to the columella (Figs. 7, 8).

The Origin and Development of the Specialized Integuments and Funiculus

The Integuments. The two integuments originate as annular ridges or girdles, adjacent to the columella (endoparietal cells) and are almost simultaneous in time of origin (Figs. 6, 7, 8). The most satisfactory basis for designating them is their region of origin and position of attachment, that is as "proximal" (outer) and "distal" (inner). The terms "inner" and "outer" apply to intermediate stages only, while the terms "proximal" and "distal" apply equally at all stages of integumentary development. The distal integument is the product of early periclinal divisions of the dermatogen (outer tunica), followed by rapid anticlinal divisions of the outer layer (Figs. 7 to 10). The resulting extensive two-layered fold (Figs. 10, 12) becomes thickened by periclinal divisions, especially in the region adjacent to the micropyle during the later stages (Figs. 13, 14, 16). The proximal integument is similar except that it has an additional hypodermal (inner tunica) component (Figs. 8, 9) which is most marked at the early stages of growth and which continues until maturity in the adfunicular portion of the flexed ovule to form a massive intrusion resulting from divisions in three planes. The outer protruding lip of the proximal integument originates by anticlinal divisions of the dermatogen and at later stages becomes thickened by periclinal divisions (Figs. 12, 13, 14, 16).

At the archesporial stage the ovule is erect (orthotropous) (Figs. 4 to 7), during meiosis it becomes pendent upon an elongated, terminally flexed funiculus (anatropous) (Figs. 8 to 10), and during the development of the

female gametophyte the ovule itself becomes flexed or geniculate (campylotropous) (Figs. 13, 14). At first the curvature is due to inequalities in the rates of division in different regions of the funiculus (Figs. 9, 10) and later is a result of marked variations in growth rate within the nucellus and the integuments (Figs. 13, 14). For facility of reference the *portion* of the flexed ovule between the micropyle and the funiculus may be designated *endoflexial*, the outer or peripheral portion as *exoflexial* and the wings as *latiflexial* (Figs. 10 to 14, 20 to 22).

The distal integument grows more rapidly in the endoflexial region to form an endoflexial lip, and the proximal integument forms an exoflexial lip in a similar manner, that is, the base of the nucellus becomes enclosed by two integumentary sheathes while the apical portion is protected by a pair of lips, different in origin and development; the distal integument is in contact with the exoflexial region of the nucellar apex, the proximal with the endoflexial region (Figs. 10 to 14, 20 to 22). The micropyle, consequently, is not a cylindrical tube surrounded by the inner integument; it is a doubly crescentic and marginally lobed passage between the exoflexial lobe of the proximal integument and the endoflexial lobe of the distal integument. Median sections at right angles to the plane of flexure show similar latiflexial portions of the distal integument enclosed terminally by the exoflexial lip of the proximal integument (Fig. 12). In this region a section of the micropyle is "I"-shaped. Meanwhile, the endoflexial portion of the proximal integument becomes very much thickened subdermally, and the resulting intrusion is chiefly responsible for the endoflexial curvature of the nucellus and of the embryo sac (Figs. 13, 14).

After the early histogenesis of the integumentary girdles there is a very minor subdermal meristematic activity, except in the endoflexial region of the proximal integument; in this region a mass of cells 20 or more units in thickness is developed by frequent divisions in both periclinal and anticlinal planes, giving rise to a knob-like intrusion into the central region of the flexed ovule. Meanwhile rapid anticlinal divisions of the exoflexial portion of this proximal integument increases the outer surface. The campylotropous ovule is an expression of these modifications of integumentary development (Figs. 13, 14). The great proportion of the integumentary sheathes and terminal lobes originates from the dermatogen. The cells first divide anticlinally to form folds two cells in thickness throughout (Fig. 10); later, secondary divisions, some periclinal, result in more massive portions, especially in the regions of the terminal lips (Figs. 13, 14, 20 to 22).

The notable curvatures of the embryo sac, of the funiculus, of the micropyle, and of other parts of the ovule, about a central massive, endoflexial portion of the distal integument are significant features of development in the campylotropous ovule.

The Funiculus. The nucellus, the integuments, and the axial row, including the sporogenous group and the columella, are entirely tunicate (epidermal and

hypodermal) in origin; in contrast, the *funiculus contains a central region that originates from the primary corpus and develops vascular tissue.*

The archesporial cells and surrounding tissue are embedded (Figs. 1, 2), and the inner cells of hypodermal origin remain below the level of the general surface during the early development of the ovular papillae (Figs. 2, 3, 17), that is, until meiosis begins in the gynospore. During spore production and the development of the functional gynospore, a rapid elongation of the funicular stalk occurs; at *first* the growth is *axially symmetrical*, giving the ovule an erect orthotropous aspect; *later* unilaterally rapid elongation results in a *flexure of the funiculus* and the ovule assumes an anatropous position (Figs. 1, 8, 9, 10, 12, 20, 21). During the development of the female gametophyte there is little or no extension of the funiculus; meanwhile the axial elongation of the nucellus and the development of the integuments, especially the endoflexial portion of the proximal integument, results in the *flexure of the nucellus* and of the embryo sac. The ovule finally takes the infolded or campylotropous posture (Figs. 14, 21, 22).

The histogenesis of the funiculus is distinct from that of the ovule proper (gynosporangium), the flexures and the fusion of parts, however, result in a structural unit that is ordinarily known as the ovule (Figs. 1, 10, 13, 14, 20 to 22). The preovular margins of the infolded carpel are four cells in thickness; periclinal divisions of the hypodermal layer on the abovular side give *five layers* of which *one is central* and may be regarded as *representing the corpus*; on the ovular side, periclinal divisions of the hypodermal layer are suppressed except at the insular regions where repeated periclinal divisions feature development of the gynosporangium. Following the production of an axial row of gynospores and of the exoparietal and endoparietal cells, divisions proceed rapidly, affecting the cells of the corpus adjacent to the basal endoparietals, and thereby the funicular axis is produced. Mitoses are both periclinal and anticlinal; the predominance of the latter results in the axial elongation of the funiculus. Periclinal divisions of the epidermis and hypodermis keep pace and complement the axial development. Ordinarily a single row of spiral vessels is differentiated at the proembryo stage. It is notable that the endoparietal cells of hypodermal origin, which are continuous with the central cells of the funiculus, remain parenchymous, and those adjacent to the embryo sac finally develop gland-like extensions into the antipodal region of the sac (Figs. 13, 14).

Development of the Female Gametophyte

Several investigators, i.e., Martin (16), Reeves (18), and Cooper (7), have described the development of the female gametophyte in *Medicago sativa* L. The development of the British Columbia hybrids follows the same general course, with a few modifications. The gametophyte is *monosporic* (15), originating from the proximal of the four gynospores; the other three disintegrate soon after being formed (Fig. 9). In abnormal ovules more than

one gynosporocyte may produce an additional spore or spores from which temporary gametophytes are initiated. When this occurs it is very evanescent.

The normal female gametophyte finally consists of *eight units*; the egg and two synergids, three antipodals, and two polar nuclei. In the gynospore, three successive free nuclear divisions occur; after the first, the common mass of cytoplasm is dense, spheroid, approximately 40μ in diameter, with abundant starch, with a large central vacuole, and with a somewhat lenticular nucleus at each pole (Fig. 10). After the third nuclear division the central vacuole is still definite in outline but the cytoplasmic mass has increased in volume, its length now being $\pm 70\mu$. Four nuclei are situated at each pole; two nuclei move and approximate each other, lateral to the vacuole; membranes delimit cytoplasmic units around each of the other nuclei while the two median nuclei and the vacuole occupy the residual cytoplasm (Fig. 12). The very rapid elongation of the nucellus and integuments and the enlargement of the *gametophytic chamber* (embryo sac) to a length of $\pm 500\mu$ follows directly. Meanwhile the original vacuole becomes lobed and ultimately forms a network of branched channels; the denser portion of the cytoplasm now occupies the axial region (Figs. 13, 14).

The egg which has finely vacuolate cytoplasm moves to a position proximal to the synergids. The combined synergids form a pear-shaped mass with a median fissure and soon their cytoplasm begins to produce irregular masses of disintegration products; the nuclei are finely granular and inconspicuous and the nucleoli disappear. The polar nuclei fuse near the distal trisect region of the gametophytic chamber (Fig. 14); their apparent movement is explained by the elongation of the sac and the polar interposition of vacuoles; the evidence indicates that the proximal portion of the ovule elongates more rapidly than the distal region and it is suggested that the autokinetic movement of the polar nuclei does not traverse a space much greater than the 20μ that formerly separated the two (cf. Figs. 12, 13). The antipodal cells elongate in accompaniment with the actively elongating sac and appear to invade the proximal third of the gametophytic chamber; they become highly vacuolate and finally disintegrate (Figs. 13, 14). The female gametophyte is now mature.

At the final stage described above one or more male gametophytes invade the gametophytic chamber, the advancing pollen tubes being simple at first, and later becoming branched. The two sperms are elongated; each has spirally-coiled, collateral bands, one of cytoplasm containing refractive globules and the other of densely staining nucleoplasm (Figs. 14, 15). The tube nucleus is very active and approaches the egg. The tube nucleus resembles the nucleus of the egg very closely, and the former may be definitely identified only after repeated observations of the various stages. Its final position in a single pollen tube in the presence of two sperms gives the most direct evidence of its identity (Fig. 15). At this stage the tube nucleus is adjacent to the egg, the zygotic sperm follows, and the other sperm advances

toward the fusion nucleus. The development of the male gametophyte and the process of fertilization are to be described in another paper.

The British Columbia hybrid plants show many deviations from the normal progress of the female gametophyte. The occurrence of two successive free nuclear divisions at the distal pole may result in a four-celled gametophyte, which includes an egg, two synergids, and a remaining cell containing a single polar nucleus which may persist or may disintegrate. Or the normal first mitosis and separation of nuclei may be followed by two mitoses at the distal pole forming the four nuclei, and by one mitosis at the proximal pole forming two nuclei only. In the latter, the development appears normal, except for the limitation of the usual number of antipodals to one. Further description of these phases of arrested development is planned in an account of abnormalities contributing to low seed production.

Development of Nutritive Tissues and Food Storage Tissue

The importance of a balanced food supply and of food utilization by the embryo, the endosperm, and the integuments has been emphasized by Brink and Cooper (2, 3). The sporogenous and gametogenous stages are equally significant, and probably no stage equals the female gametophyte in rate of enlargement and consequent requirement of food material. Food is most readily available in true or in colloidal solution; the most active stages are characterized by vacuolate or finely granular cytoplasm, the interpauses, by stored food products. Alternate stages of food storage and utilization are recognized as featuring progressive periods of growth.

During sporogenesis the columella, composed of endoparietal cells, is the chief medium of communication between the ovule and the carpel wall. The cells of the columella are parenchymous and contain a dense cytoplasm; their development precedes the early gametophyte which partly uses their food store. Later the columella becomes conductive and its glandular basal cells protrude into the gametophytic sac (Figs. 10, 12, 13, 14).

At the time of the first division of the functional gynospore this cell contains an abundant food supply as evinced by the numerous marginal starch grains and granules surrounding the large, central vacuole. The nearly complete reabsorption of the three sister gynospores indicates the source of a considerable proportion of this supply (cf. Figs. 9, 10). Most of the food is utilized during the mitotic division and wall-forming stages of gametophyte development. A very small amount of starch remains in the cytoplasm containing the free nuclei when the six polar cells are delimited. The new cells have a dense, colloidal food-containing cytoplasm and marked nucleoli (Fig. 12).

The rapid enlargement in the gametophytic chamber synchronizes with the partial digestion of the three- to four-layered wall of the nucellus, with the elongation of the columella cells and the proliferation of the gland-like cells into the embryo sac, with the differentiation of conductive tissue in the funiculus, and last, with the disintegration and digestion of the antipodal cells and synergids. The resulting gametophytic chamber is filled with

vacuoles containing soluble food and masses of starch grains, especially in the vicinity of the egg and fusion nucleus (Figs. 13 to 15). The cytoplasm of the egg and of the pollen tube are locally vacuolate or finely granular.

At the time of fertilization the nucellus is entirely disintegrated at the micropylar region whereas one or two cell layers of the antipodal portion usually remain intact. The marked accumulation of stored food within the chamber at the final stage of gametophytic development would appear to originate chiefly from the tapetal-like nucellar jacket and this residual store provides at least partly for the proembryo and early endosperm. The water supply used by the ovule is, probably, derived directly through the columella and the vascular tissue of the funiculus. In view of later developments and competition for the food supply, as emphasized by Brink and Cooper (3), the degree of development of the various parts and their proportionate food supply, at this late gametophytic stage, are significant features in the normal production of seeds. In the British Columbia hybrids the proliferation of the endoflexial portion of the integument normally occurs before the origin of endosperm, and thereby the competition that otherwise would result in "somatoplastic sterility" is reduced or obviated.

Fertilization initiates a series of changes not only in the meristematic development of the embryo and of endosperm cells, but also in the specialization of protective and local storage cells of the integuments (3, 8, and Fig. 16). Normal development of the seed is dependent upon balanced development in these preliminary stages and it is during this critical period that certain of the ovules degenerate most markedly, a significant proportion ceasing development at one of the proembryo stages.

The aim of this account has been to trace the normal development and to designate a standard that may be used in judging the successes and the failures of ovule development in *Medicago*. There are planned further papers that demonstrate the importance of timing in ovule and pollen tube growth, and of the synchronous initiation of zygote and of primary endosperm as factors in the harmonious balance of processes essential for seed production.

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EXPLANATION OF FIGURES

Figs. 2 to 21 were drawn with the aid of a camera lucida. Figs. 17 to 22 are reconstructions from the analyses of microscopic sections. Figs. 1 to 16 reduced two-thirds in reproduction; Figs. 17 to 22, ca. two-fifths.

LEGEND

- (a) Cells of the embryo sac and their precursors, i.e., archesporial cells, gynospores, cells of the female gametophyte, the disintegrating cells of the inner nucellus, cells of the embryo and of the endosperm, all are completely stippled.
- (b) Exoparietal and endoparietal (columella) cells of the axial row have nuclei completely stippled and cytoplasm shown by scattered dots.
- (c) Cells of the funicular axis (preconductive) have nuclei completely stippled.
- (d) Differentiated conductive cells have spiral bands.
- (e) Cells of the nucellus have outlines of the nuclei and nucleoli only.
- (f) Cells of the distal integument have nuclear outlines only.
- (g) Cells of the proximal integument have cytoplasmic membranes stippled.
- (h) Cells of the differentiating epidermis, after fertilization, have granules at the inner and outer margins.
- (i) All other cells, including peripheral layers of the funiculus, have outlines of walls only.
- (j) In Fig. 1 the epiphore (calyx tube or receptacle cup) is shown in outline; the petals have additional marginal stippling; stamens contain pollen grains; the carpel is dotted and the embryo sac is fully stippled; all vascular bundles are enclosed and larger bundles are half-hatched.

DESCRIPTION OF SPECIAL FEATURES OF FIGURES

FIG. 1. Transverse section of the flower at the time of the two-celled gametophyte, at the anther level of the longer stamens; the folded carpel, with separate funiculus and nucellus of adjacent ovules; five stamens; five filaments (stippled lines) representing the position of the shorter stamens; five separate petals and the epiphore (calyx tube or receptacle cup). 40X.

FIGS. 2, 3. Sections of carpel wall at the marginal fold, showing primordia of the ovules at two stages.

FIG. 2. General periclinal divisions of the hypodermal layer on contraovular side; localized periclinal divisions giving rise to the secondary gynosporangial (archesporial) cell and the endoparietal initial. 360X.

FIG. 3. Several potential gynosporangial tiers, one tier forming the axial row consisting of a tertiary gynosporangial cell (gynosporocyte), the dividing endoparietal cell, and an exoparietal initial. 360X.

FIGS. 4 to 10. Sections of the ovular primordium and later the developing ovule, with the funiculus, in the plane of the carpel's longitudinal axis. This plane also shows the flexure of the funiculus and of the ovule and the asymmetrical growth of the integuments.

FIG. 4. A single axial row, a gynospore, a single exoparietal, and two endoparietal cells (columella). 360X.

FIG. 5. Similar, with three endoparietals. Lateral tiers of potential archesporial cells are forming the inner nucellus. 360X.

FIG. 6. Three tiers of active archesporial cells each with a sporocyte, an exoparietal, and three or four endoparietal cells. 200X.

FIG. 7. Similar, the secondary sporocyte stage; axial row somewhat asymmetrically situated; primordia of the distal integument, derived chiefly from dermatogen (outer tunica). 360X.

FIG. 8. Primordia of proximal integument, partly hypodermal in development; second meiotic division; axial and transverse divisions of the endoparietal cells produce a sectionally double-tiered columella. A 90-degree funicular flexure results in a pendent ovule. 360X.

FIG. 9. Similar, and somewhat more advanced; the two proximal gynospores, with dense protoplasm, and axially situated; the two distal gynospores (one shown), disintegrating and obliquely placed. 360X.

FIG. 10. A funicular flexure through 180 degrees results in an anatropous ovule; the exoflexual extension of the distal integument is marked; a binucleate female gametophyte, with large central vacuole. 360X.

FIG. 11. A tetranucleate female gametophyte. 430X.

FIG. 12. A section of the nucellar portion of an ovule (funiculus omitted) in a plane transverse to the flexed axis of the carpel (cf. Fig. 1). The compacted, seven-celled stage of the female gametophyte: egg, two synergids, three antipodals, central cell containing two polar nuclei, the vacuole extending and branching. The exoflexual fold, only, of the distal integument appears. 360X.

FIGS. 13, 14. Sections of the ovule in the plane of the longitudinal axis of the carpel showing the flexures of the campylotropous ovule.

FIG. 13. A greatly elongated gametophytic sac occasioned by the anticlinal divisions of the wall cells, especially the exoflexual. Micropyle surrounded by the exoflexual lobe of the proximal integument and the endoflexual lobe of the distal integument; the distal integument enclosing the basal portion of the nucellus only, on the exoflexual side; the proximal integument becomes massive at the centroflexual region. Funiculus with vascular strand; glandular, hair-like endoparietals; exoparietals lenticular; disintegrating nucellar cells; antipodals suspended in the sac; synergids disintegrating; polar nuclei approximated; tube nucleus in contact with the egg. 165X.

FIG. 14. Similar and further developed, immediately preceding fertilization. Distal portion of nucellus completely disintegrated; fusion nucleus; rich food store and extreme vacuolation within gametophytic sac. Spiral sperm lateral to synergids. 165X.

FIG. 15. The pollen tube, containing the tube nucleus and two spiral sperms, advancing by the side of the egg, with lobes approaching the fusion nucleus (latter not shown); synergids disintegrating; cytoplasm of egg and pollen tube vacuolate and surrounded by abundant starch. Nuclei of egg and tube containing densely staining nucleoli. 930X.

FIG. 16. Section of micropylar portion of the ovule, transverse to the longitudinal axis of the carpel; the filamentous proembryo and free nuclear endosperm. Epidermis of the proximal integument differentiated and containing abundant globules, the distal integument and nucellus discontinuous, distally. 165X.

FIGS. 17 to 22. Diagrams to illustrate the development of the campylotropous ovule of *Medicago*, and particularly the relationships of the funiculus, the integuments, the nucellus, and the gametophytic sac. Figures on the left are "translux" diagrams, on the right are surface diagrams, except that the positions of the nucellus, the gametophytic sac, and the vascular bundle are indicated by series of dots. ca. 90X.

FIG. 17. The ovular mound or primordium.

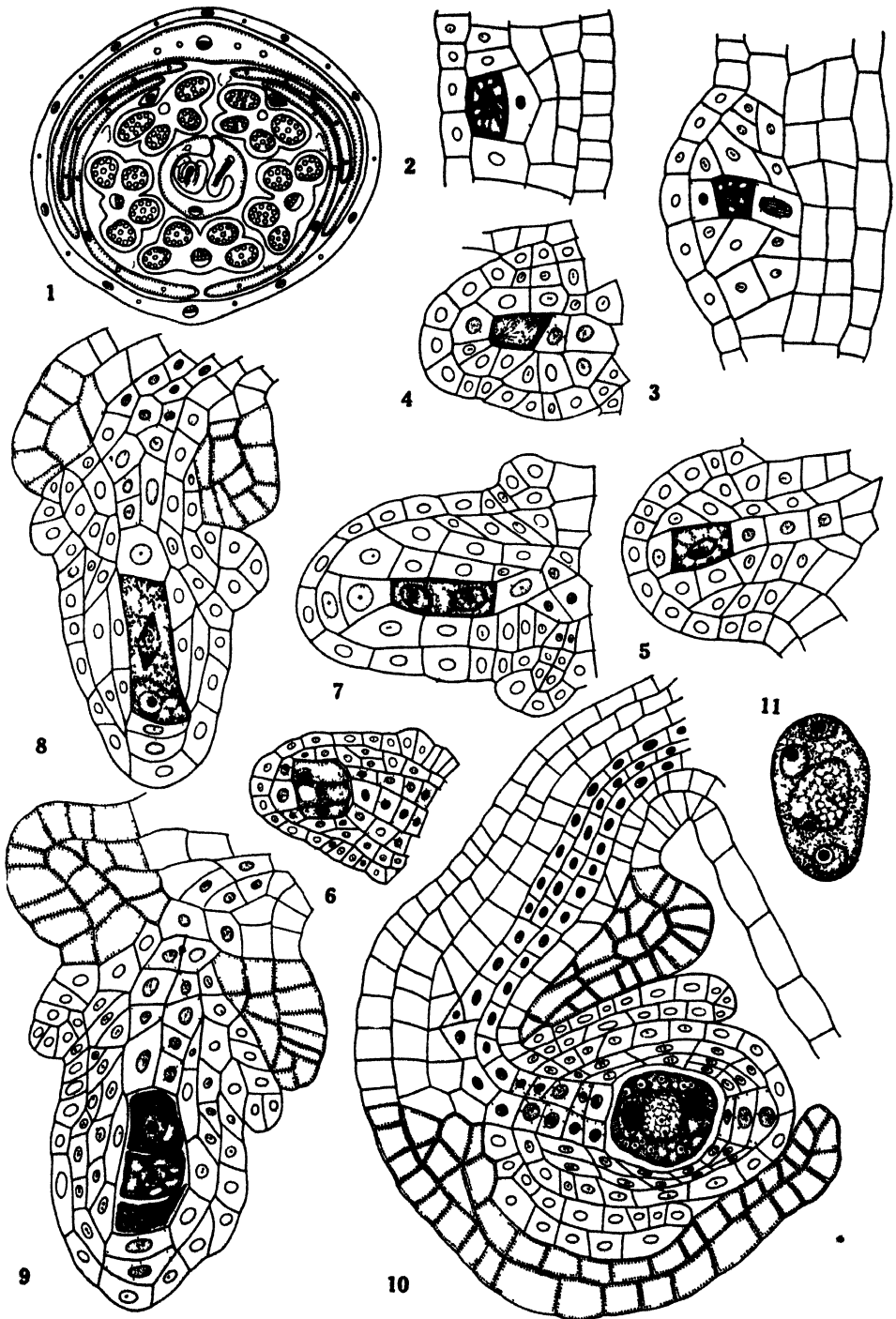
FIG. 18. Nucellus exposed; funiculus elongating; distal integument initiated; axial row, including gynospores, developing; columella complete.

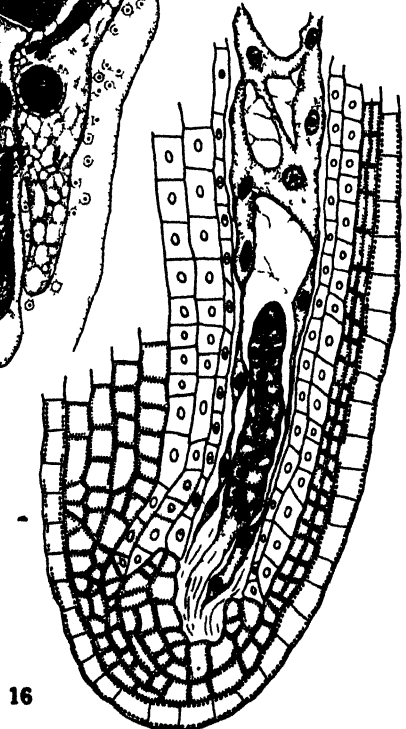
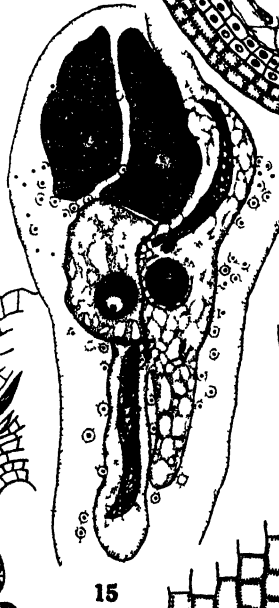
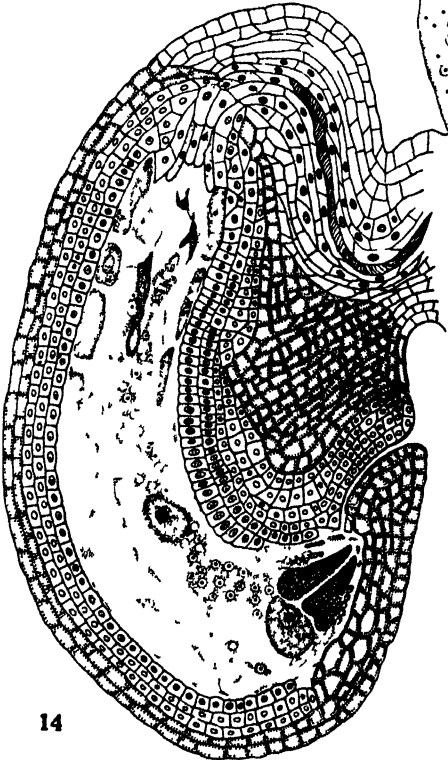
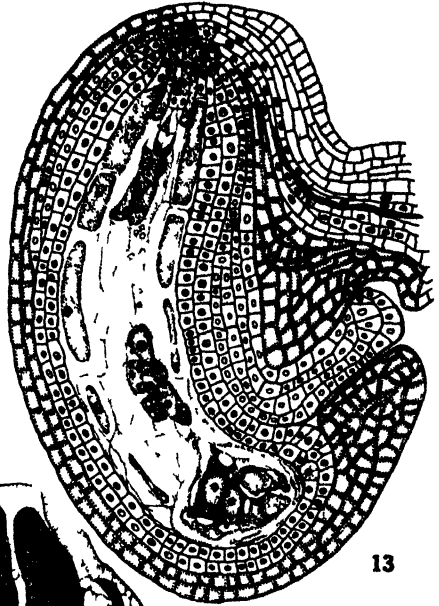
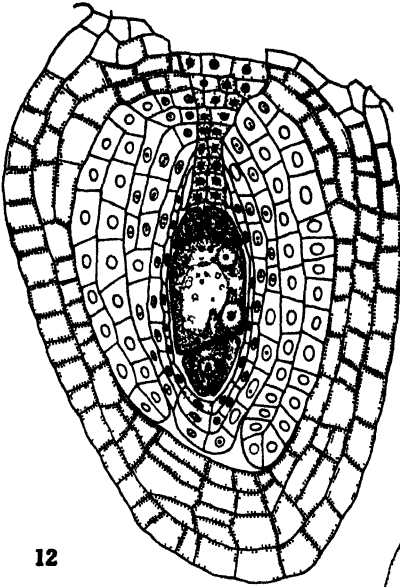
FIG. 19. Flexure of funiculus begins, proximal integument forms an exoflexual lip, proximal gynospore differentiates.

FIG. 20. Funicular flexure, 135 degrees, exoflexual lip of the proximal integument and endoflexual lip of the distal integument nearly enclose the nucellus; seven-celled, female gametophyte retains the central vacuole and mound form.

FIG. 21. *The arc flexure of the funiculus complete; the vascular strand differentiating; the integumentary lips enclose the nucellus except for a sectionally doubly-crescentic micropyle; the exoflexial lip of the proximal integument nearly encloses the lip of the distal integument; the endoflexial portion of the proximal integument becomes massive and occasions the flexure of the elongating gametophytic sac; female gametophyte mature; glandular development of distal cells of columella.*

FIG. 22. *The sigmoid flexure of the funiculus accompanies a marked proliferation of the proximal integument in the centrofexial region; the filamentous proembryo and the free nuclear endosperm invade the crescentic embryo sac of the campylotropous ovule.*





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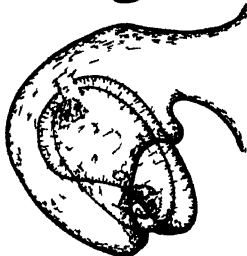
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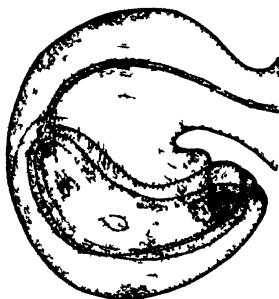
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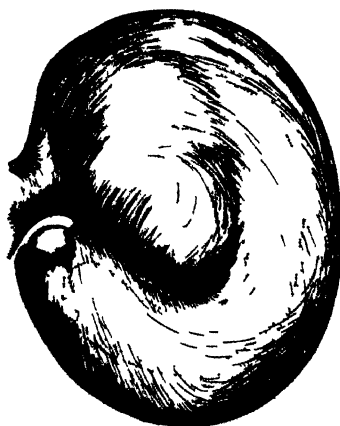
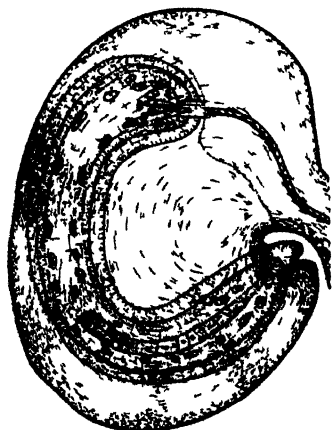
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THE EFFECT OF HIGH TEMPERATURE ON THE STEM RUST RESISTANCE OF WHEAT VARIETIES¹

BY T. JOHNSON AND MARGARET NEWTON²

Abstract

Eighteen stem rust resistant wheat varieties were tested, in the greenhouse, for their reaction to three physiologic races of *Puccinia graminis Triticum* Erikss. and Henn. at three different temperatures: a constant low temperature of about 60° F., a constant high temperature of about 80° F., and an intermediate temperature which fluctuated daily from 50° to 55° F. at night to 70° to 85° F. at midday.

At the low and at the intermediate temperature some of the varieties proved immune while others proved highly or moderately resistant. At the high temperature five varieties (Bokveld, Iumillo, Gaza, Red Egyptian, and N.A. 95 Egypt) were immune or highly resistant; six varieties (Marquillo × Waratah, Hope, Hochzucht, Minor, Bobin Gaza Bobin, and Federation × Acme) were moderately resistant; and seven varieties (Kenya, Syria, McMurachy, Sweden, Rhodesian, Talberg, and Eureka) were moderately or completely susceptible.

Introduction

It is well known that temperature influences the stem rust reaction of the wheat plant. This fact has been clearly demonstrated by several investigators in studies on the rust reaction of wheat seedlings. Waterhouse (11) and Johnson (5) showed that the indeterminate (x) reaction which certain varieties display at moderate temperatures is replaced by resistance at low and susceptibility at high temperatures. Melander (8) has shown that temperatures slightly above freezing largely suppress stem rust development and Johnson and Newton (6) have shown that excessively high temperatures induce resistance, at least to some physiologic races.

Less attention has been given to the influence of temperature on the stem rust reaction of the wheat plant in more advanced stages of growth. It has, however, been shown recently (9, 10) that certain strains of *vulgare* wheat from Kenya, East Africa, and the Canadian variety McMurachy, which are immune or nearly so at low and moderate temperatures, become partially or completely susceptible at high temperatures in both the seedling and the adult stage. Somewhat similar results had previously been reported for the variety Hope (7) which proved less resistant at 75° to 80° F. than at lower temperatures. The studies on the influence of temperature on reaction to stem rust were extended to include a group of wheats from a large collection made by Mr. S. L. Macindoe of Sydney, Australia. The varieties tested were selected from a larger number grown in 1940 on the plots of the Dominion Rust Research Laboratory where they were exposed to natural infection

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plus an artificially produced stem rust epidemic. In addition to the varieties selected from Mr. Macindoe's collection, there were also included in these tests the resistant varieties McMurachy, Kenya R.L. 1373, Hope, and Iumillo, and, for purposes of comparison, the susceptible variety Marquis. With the exception of Marquis and Eureka, all the varieties selected for the temperature studies had shown immunity or near-immunity in the field tests.

The Kenya strains and McMurachy have, since they were first grown at Winnipeg in 1934 and 1935, respectively, proved immune to stem rust or nearly so. In 1938, it was brought to the attention of the plant breeders at Winnipeg that these varieties were not immune and sometimes not even highly resistant when grown at St. Paul, Minn., Manhattan, Kansas, and some other points in the United States. It seemed probable therefore that their rust reaction was being influenced by the environment in which they were grown. The first proof that their resistance disappeared at high temperatures came as a result of experiments conducted by the writers in an attempt to explain certain unexpected infection results obtained in progeny tests carried out by Dr. R. F. Peterson in the winter 1939-1940. He found that seedlings of Kenya, McMurachy, and some of their progeny, known to be immune in the field, proved at times to be only moderately resistant or even susceptible when tested in the greenhouse. At his request an investigation was undertaken. Experiments in which light and temperature were kept under control soon proved that high temperature was the chief factor responsible for the breakdown of seedling resistance. Subsequent experiments, as already mentioned, have shown that the resistance of adult plants may also be broken down at high temperatures.

Methods

Three physiologic races with distinctly different infection characteristics were selected for the tests, namely, races 56, 29, and 15. Race 56 is, at present, the predominant physiologic race of wheat stem rust in North America. It attacks many *vulgare* wheats severely, but is not highly pathogenic on most *durum* and emmer wheats. Race 29 attacks many *vulgare* wheats heavily, *durum* wheats moderately, but emmer wheats lightly. Race 15 attacks all three groups of wheat rather severely.

The test plants were grown to the heading stage in 6-in. flower pots—four plants to each pot. At heading or immediately after, the inoculum was applied to leaves, stems, and peduncles with the fingers, after which the plants were kept for 24 hr. in damp chambers consisting of a framework of wooden laths covered with heavy cardboard which, when soaked with water, maintained a satisfactory film of moisture on the plants. Infection took place at a moderate temperature, but when the plants were removed from the inoculation chamber four plants inoculated by each physiologic race were placed in each of three greenhouses maintained at different temperatures. Two of these greenhouses were thermostatically controlled. These were kept respectively at around 60° and 80° F. In the third greenhouse the temperatures fluctuated daily from 50° to 55° F. at night to 70° to 85° F. at

TABLE I

REACTION IN GREENHOUSE TESTS, OF 19 WHEAT VARIETIES IN THE HEADING STAGE TO THREE PHYSIOLOGIC RACES OF WHEAT STEM RUST AT LOW, MEDIUM, AND HIGH TEMPERATURES (RUST PERCENTAGES IN PARENTHESES)

Variety	1st Test—December to March							
	Date mcc.	Temp., °F.	Race 15		Race 29		Race 56	
			Leaves	Sheaths	Leaves	Sheaths	Leaves	Sheaths
Iumillo R.L. 7*	Jan. 27	60 3 63 5 81 5	I — —	I — —	I — I	I — R	I I I	I I I
Gaza W.L.W.-S. 755**	Dec. 2	61 3 63 1 81 0	I — I	I — I	I — I	I — I	I — I	I — I
Bokveld C. 12080-S. 656								
Red Egyptian C. 12081-S. 979	Jan. 17	61 2 60 9 82 6	I — R	I — R	I — R	I — R	I I R	I — R
N.A. 95 Egypt C. 12095-S. 674	Jan. 9	60 1 61 0 83 2	— — R	I — R	I — R	— — I	I I R	I — R
Marquillo × Waratah S. 906	Dec. 6	61 7 63 7 82 3	I — I	I — —	I — I	I — I	I I I	I I I
Hope R.L. 209	Feb. 12	60 5 64 2 80 6	MR — MR	MR — MR	R — MR	MR — MR	R I R	I I I
Hochzucht C. 10847-S. 765	Jan. 22	60 2 72 8 80 7	R — R	R — R	R — I	R — I	I I MR	R I MR
Minor C. 9142-S. 931	Feb. 13	60 8 63 9 80 4	R — R	R — R	R — MR	MR — MR	— R MR	— R R
Bobin Gaza Bobin S. 203								
Federation × Acme S. 630	Feb. 4	62 4 62 4 80 2	R — R	R — R	R — R	R — R	R R R	R R R
Syna C. 10349-S. 1027	Feb. 18	60 6 64 9 80 7	I — MS	I — MS	I — MS	I — S	I I MS	I I S
McMurachy R.L. 1313	Jan. 17	61 2 63 1 82 9	I — —	I — —	I — S	R — S	I I MS	I — S
Kenya R.L. 1373	Feb. 3	59 5 64 7 81 0	I — MS	I — S	I — MS	I — MS	I I MR	I I MS
Sweden C. 12266-S. 1038	Jan. 8	60 3 60 7 83 0	I — MS	I — MS	— — S	— — MS	I I S	I — MS
Rhodesian TRS No. 8-S. 971	Jan. 23	60 1 72 7 80 7	I — S	I — S	I — S	I — S	I I S	I I S
Talberg C. 12261-S. 1056	Feb. 7	60 0 63 5 80 0	I — S	I — S	I — S	I — S	I I S	I I S
Eureka 2 C. 12890-S. 723								
Marquis R.L. 572	Jan. 29	61 0 63 4 82 1	MS — S	S — S	S — S	S — S	MS S S	S S S

NOTE. I = immune; R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible; tr = trace.

* Dominion Rust Research Laboratory accession number.

** Number under which the variety was received from Mr. S. L. Macindoe.

TABLE I—*Concluded*

REACTION IN GREENHOUSE TESTS, OF 19 WHEAT VARIETIES IN THE HEADING STAGE TO THREE PHYSIOLOGIC RACES OF WHEAT STEM RUST AT LOW, MEDIUM, AND HIGH TEMPERATURES (RUST PERCENTAGES IN PARENTHESES)—*Concluded*

Variety	2nd Test—March to May							
	Date inoc.	Temp., °F.	Race 15		Race 29		Race 56	
			Leaves	Sheaths	Leaves	Sheaths	Leaves	Sheaths
Iumillo R.L. 7*	April 25	63 4 69 2 78 4	I I I	I I I	I I I	I I I	I I I	I I I
Gaza W.L.W.-S. 755**	Mar. 18	61 5 66 7 81 3	I I I	I I I	I I I	I I I	I I I	I I I
Bokveld C. 12080-S. 656	May 1	68 3 69 9 77 2	I I I	I I I	I I I	I I I	I I I	I I I
Red Egyptian C. 12081-S. 979	April 24	65 5 68 9 78 4	I I R(tr)	I I R(10)	I I R(tr)	I I R(tr)	I I R(tr)	I I I
N.A. 95 Egypt C. 12095-S. 674	Mar. 27	63 5 66 6 80 2	I R(tr) R(tr)	I R(tr) R(tr)	R(tr) R(tr) R(tr)	R(tr) R(tr) R(tr)	R(tr) R(tr) R(tr)	R(tr) R(tr) R(tr)
Marquillo × Waratah S. 906	Mar. 24	64 2 64 5 83 6	R(tr) I MR(tr)	MR(tr) I MR(tr)	I I I	I I I	R(tr) I MR(3)	R(tr) R(tr) MR(1)
Hope R.L. 209	April 28	62 6 67 9 78 8	MR(tr) R(tr) MR(tr)	MR(20) MR(15) MR(15)	MR(tr) R(tr) I	MR(5) MR(10) MR(15)	I I I	I R(tr) I
Hochzucht C. 10847-S.765	April 15	64 7 68 2 78 9	MR(30) R(20) MR(10)	MS(50) MR(40) MR(25)	R(5) R(tr) R(tr)	R(5) I I	R(1) R(5) R(5)	MR(5) MR(25) MR(5)
Minor C. 9142-S. 931	May 1	68 3 69 9 77 2	MR(25) R(25) R(20)	MR(30) R(35) MR(20)	MR(15) R(15) MR(15)	MR(40) MR(30) MR(35)	MR(20) R(25) MR(35)	MR(30) R(40) MR(45)
Hobin Gaza Bobin S. 203	Mar. 27	63 3 66 7 83 4	MR(20) MR(25) MR(15)	MR(25) MS(50) MR(45)	MR(20) MR(20) MS(25)	MR(35) MS(45) MS(50)	R(15) MR(25) MR(20)	R(25) MR(50) MR(40)
Federation × Acme S. 630	April 22	64 6 68 3 80 8	R(5) MR(5) MR(5)	R(25) MR(20) MS(20)	R(tr) MR(5) MR(2)	R(10) MR(25) MS(15)	I R(10) MR(1)	R(tr) MR(20) MR(10)
Syria C. 10349-S. 1027	May 2	69 1 71 5 76 3	I I MR(5)	R(5) R(tr) MR(45)	I I R(tr)	R(2) R(tr) MS(25)	I I I	R(tr) R(tr) MR(10)
McMurachy R.L. 1313	April 29	65 8 69 3 80 3	R(tr) R(2) MS(5)	R(tr) MR(20) MS(10)	R(tr) I S(5)	MR(tr) R(2) S(10)	R(tr) R(2) MS(3)	R(tr) MR(15) MS(2)
Kenya R.L. 1373	April 28	62 6 67 9 78 8	I R(2) MS(10)	I R(10) MS(40)	I MR(tr) MS(10)	I R(tr) MS(25)	I R(tr) MR(5)	I R(5) MR(15)
Sweden C. 12266-S. 1038	April 21	63 7 67 9 78 6	I MR(10) MS(2)	MR(tr) MR(25) MS(20)	I R(5) S(10)	R(tr) MR(20) S(45)	I R(1) MS(15)	R(tr) MR(15) MS(30)
Rhodesian FRS No. 8-S. 971	April 23	64 3 69 0 79 5	I R(tr) S(2)	I R(tr) S(10)	I I S(2)	I R(tr) S(10)	I R(tr) S(2)	I R(tr) S(5)
Talberg C. 12261-S. 1056	April 29	65 8 69 3 80 3	R(tr) MR(3) S(5)	R(tr) MS(15) S(10)	R(tr) R(tr) S(tr)	MR(5) MR(20) MS(5)	MR(tr) S(tr)	MR(tr) MR(5) S(1)
Eureka 2 C. 12890-S.723	Mar. 27	63 3 66 7 84 3	R(2) MR(5) MS(30)	MR(5) MR(25) S(60)	I R(tr) S(40)	R(tr) MR(3) S(65)	I MR(5) MS(30)	R(5) MR(20) S(80)
Marquis R.L. 572	April 24	65 5 68 9 78 4	S(20) S(25) S(10)	S(50) S(70) S(50)	S(20) S(25) S(25)	S(55) S(50) S(55)	S(25) S(30) S(30)	S(65) S(65) S(60)

NOTE. I = immune; R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible; tr = trace.

* Dominion Rust Research Laboratory accession number.

** Number under which the variety was received from Mr. S. L. Macindoe.

midday. It should be noted that the mean daily temperatures expressed in Table I were calculated from thermographic records at two-hour intervals.

Two successive tests were carried out on all but three of the 19 varieties studied. The first test, on plants sown September 20, 1940, was made during the winter months of December, January, February, and early March. The second test, on plants sown January 10, 1941, was conducted from the latter part of March until about the middle of May. Towards the end of this period (particularly from May 1 to May 20) the out-of-doors temperature became too high for satisfactory thermostatic control of the low temperature greenhouse. The mean daily temperature of this greenhouse therefore rose considerably towards the end of the second test.

The tests of the adult plants were accompanied by simultaneous infection tests with seedlings. All varieties were also tested at 60° and 80° F. to eight other physiologic races (races 17, 19, 21, 32, 36, 38, 48, and 152) so that some knowledge could be gained of their general rust reaction.

In estimating the degree of resistance of the adult plants personal judgment was eliminated as far as possible by recording the infection types present on each of the four uppermost leaves and sheaths and converting these into numerical values for which averages for leaves and stems could be calculated. The system used was a modification of that originally described by Goulden, Newton, and Brown (4). The infection types 0, 1, 2, 3, and 4 were given values of 1, 5, 10, 15, and 20, respectively, and the types $x=$, $x-$, x , $x+$, and $x++$ values of 11, 12, 13, 14, and 15. In determining the rust reaction of a variety, numerical values of 0 to 25 were regarded as representing immunity, 26 to 75 resistance, 76 to 125 moderate resistance, 126 to 155 moderate susceptibility, and 156 and above as susceptibility.

The Effect of Temperature on Stem Rust Reaction

Field Test

As already stated, all the varieties tested in the greenhouse were subjected, in the summer of 1940, to a field test in which they were exposed to natural stem rust infection on which was superimposed an artificially induced epidemic comprising many physiologic races. Of the 19 varieties previously mentioned only two, Marquis and Eureka 2, were severely rusted, four (Gaza, Rhodan, Kenya, and Iumillo) bore no stem rust infection, while the remaining 3 varieties showed only a trace of rust.

Greenhouse Tests on Adult Plants

The two tests with adult plants recorded in Table I were conducted under approximately the same conditions of temperature. Conditions of light, however, were not similar owing to the fact that the first test was conducted in midwinter, whereas the second was carried out in the spring. It should be

PLATE I



*Reaction of the uppermost sheath of Eureka 2 to *P. graminis* Triticum race 56 at three different temperatures: left, at 63.3° F.; centre, at 66.7° F.; right, at 84.3° F.
At fluctuating temperatures the largest pustules are directly above each node.*

noted, too, that the first test was less comprehensive inasmuch as only race 56 was used in the infection studies at moderate (fluctuating) temperature.

Table I gives, for each variety, the mean reaction of leaves and sheaths as determined by infection type and also, for the second test, the amount of rust, expressed in percentages. The percentage figures, as well as the reaction symbols, are indicative of the degree of resistance, but undue emphasis should not be placed on the percentages because of possible variation in the amount of inoculum applied and conditions of temperature and moisture at the time of infection.

On the basis of response to temperature the varieties may be divided into two groups: those whose reaction is little influenced by temperature, including Gaza, Bokveld, Iumillo, Red Egyptian, N.A. 95 Egypt, Marquillo \times Waratah, Hope, Hochzucht, Minor, and Bobin Gaza Bobin; and those whose resistance is more or less broken down at high temperatures, including Federation \times Acme, Syria, McMurachy, Kenya, Sweden, Rhodesian, Talberg, and Eureka. The last six varieties were the ones most markedly affected by temperature. Marquis, which was included in the tests only for comparison, was equally susceptible at all three temperatures. Within each of these two groups the varieties are arranged in Table I in approximate order of resistance. Plate I shows the reaction of Eureka at three different temperatures.

A few discrepancies between the first and second tests may be noted. Marquillo \times Waratah proved immune at all three temperatures in the first test but was in some cases only moderately resistant in the second test. Hochzucht was somewhat less resistant in the second test than in the first, and Federation \times Acme was more susceptible at the high temperature in the second test. In most of the varieties, however, agreement between the two tests is reasonably close.

Greenhouse Tests on Seedling Plants

All the varieties except Bokveld were tested in the seedling stage at 60° and 83° F. for their reaction to physiologic races 15, 17, 19, 21, 29, 32, 36, 38, 48, 56, and 152. In general, the seedling response to temperature of each variety resembled that of the adult plants closely. Two varieties, however, proved exceptional, namely, Marquillo \times Waratah, whose resistance to races 15 and 56 broke down at the high temperature in the seedling stage but not in the adult stage, and Minor, whose seedlings were somewhat more susceptible than the adult plants at both temperatures. The variety Hope differed from the other varieties tested in that it was susceptible in the seedling stage to several races to which it has mature-plant resistance.

At the low temperature the following varieties proved either immune or highly resistant to the eleven above-mentioned races: Iumillo, Gaza, Bobin Gaza Bobin, Eureka, Red Egyptian, Sweden, Kenya, McMurachy, Federation

× Acme, Rhodesian, Syria, and Talberg. N.A. 95 Egypt proved moderately resistant to all the races, while Minor was moderately resistant to some and highly resistant to others. Hochzucht and Marquillo × Waratah were susceptible to races 36, 38, and 48 but resistant to others. Hope was susceptible to races 15, 17, 21, 29, and 32.

Discussion

It has become clear within the last few years that stem rust resistance of wheat varieties varies considerably according to the locality in which the varieties are grown. Those that are resistant when grown in the Great Plains region of North America are not necessarily so when grown in South America (1) or in Africa (2, 3). Such discrepancies in reaction may be due to the presence of pathogenically different strains of the rust in different regions, or they may result from differences in environmental conditions, or from a combination of both factors.

Data presented in Table I show that varieties that at low or moderate greenhouse temperatures possess about an equal degree of resistance to stem rust may vary greatly in their stem rust reaction at higher temperatures. Some, like Iumillo, Bokveld, Gaza, and Red Egyptian, maintain much or all of their high resistance at higher temperatures; others, including McMurachy, Kenya R. L. 1373, Talberg, Sweden, Rhodesian, Syria, and Eureka 2, lose most or all of their resistance when the mean temperature approaches 80° F. In the field, the last-mentioned group of varieties could not be expected to display high stem rust resistance in regions where temperatures are excessively high during the period of stem rust infection. McMurachy and the Kenya wheats are, in fact, known to have less stem rust resistance at St. Paul, Minn., and at Manhattan, Kansas, than at Winnipeg. The summer temperatures at these points are definitely higher than at Winnipeg. For the 9-year period 1932-1940 the mean temperature for July was 69.7° F. at Winnipeg, 76.7° F. at St. Paul, and 84.2° F. at Topeka, Kansas (50 miles from Manhattan). The mean temperature at Winnipeg is below that which, according to greenhouse experiments, should induce susceptibility (about 75° F.). At St. Paul the temperature is approximately at the point where some breakdown of resistance might be expected, while at Topeka, Kansas, it is definitely of the order required to bring about susceptibility. The reaction of the Kenya wheats at St. Paul in 1940 may provide a specific instance of the influence of high temperature on rust reaction. According to Watson (12), some wheat varieties including certain of the Kenya wheats were less resistant at that station than usual. In that year, the mean temperature for July was 75.8° F., whereas the mean temperature for the last 15 days of July was about 81° F. It seems probable that the unusually high temperatures that prevailed in the latter part of July influenced the stem rust reaction of the Kenya wheats.

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A PHYTOPHTHORA TOMATO DISEASE NEW TO ONTARIO¹

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Abstract

In recent years a destructive disease has affected tomato plants in certain greenhouses in central Ontario. This disease is characterized by a damping-off of seedlings and a rot of stems, fruits, leaves, and roots. The causal organism has been identified as *Phytophthora parasitica* Dast. The effects of various nutrient media, of temperature, of atmospheric humidity, and of the acidity of the medium on the growth of the fungus in culture have been studied.

An extensive investigation has been made of certain factors affecting the parasitic activity of this soil-borne pathogen. Disease is favoured by high atmospheric temperature and high relative humidity. Disease incidence also varies directly with the moisture content of the soil and is maximum at soil temperatures near 22° C. The ability of the pathogen to establish itself in soils depends upon the type of soil, upon the other micro-organisms present, and upon the substrate available for saprophytic development. The rates of invasion of non-infested soils were determined and found to be retarded by the competitive factor and accelerated by the presence of living roots of tomato seedlings. The activity of the pathogen is suppressed by the addition of soybean residue to infested soil.

The potential host range of this isolant of *P. parasitica* has been partially determined by means of artificial inoculations.

Several methods for the control of the disease were tried, and their value is discussed.

Introduction

Within recent years there has appeared in central Ontario a destructive disease affecting tomato plants in all stages of their development. This disease is not only new to this area, but seems to be distinct from any other reported on this host. Thus far the outbreaks have been strictly localized in distribution and confined almost exclusively to tomato plants grown under glass. The disease was first observed in the Experiment Station greenhouses at Vineland, Ontario, during the summer of 1937. Since then it has appeared in several of the greenhouses in this district and in one outdoor crop. Losses in some instances were very heavy.

In addition to these cases, which were confined to central Ontario, Dr. Fitzpatrick of the Dominion Laboratory of Plant Pathology at Summerland, B.C., reported in 1940 one outbreak of a similar disease in the Okanagan Valley. A comparison of the causal organisms showed the two to be identical.

The fact that new cases are reported each year seems to indicate that the disease is increasing in economic importance. Since this is the case and since there is no information available regarding the disease, it is considered desirable that the results of this investigation should be reported at the present time.

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The investigation covered several aspects of the disease. The causal organism was isolated and identified as *Phytophthora parasitica* Dast. The effects of various factors on the growth of the organism in culture were studied. With respect to epidemiology, extensive studies were made of the factors influencing the parasitic activity of the pathogen. Its ability to attack species of plants other than its natural host was also investigated. Finally, possible methods for the control of the disease were tested.

Symptoms of the Disease

The most striking aspect of the disease is its attack on the seedling stage of the host. Seedlings may be killed either before or after they emerge from the soil. The amount of pre-emergence blight can be determined by comparing the percentage germination of seeds planted in non-infested soil of the same type. Postemergence killing of seedlings results in the effect commonly called "damping-off". A seedling may be killed while emerging, before the hypocotyl becomes erect and pulls the cotyledons out of the soil. If it is attacked later, localized killing of the tissues occurs, usually near the soil line, and the plant falls over. Young seedlings collapse so quickly that several in a flat may fall over within an hour, and, under conditions favourable for disease, 50% may succumb in a single day. Seedlings that are growing in a flat of infested soil become infected not only when they are young, but continue to die, though at a decreasing rate, as they become older. When the survivors are transplanted, even into non-infested soil, there is a very high rate of mortality, amounting in some cases to 100%.

In older plants grown indoors infection is largely confined to the stems. The symptoms here might be described variously as a collar rot, stem girdle, or stem canker (Plate I, Figs. 10, 11, and 13). While in many cases the lesion is restricted to a narrow zone that girdles the stem, in other cases it extends along the stem for several inches before the plant succumbs. On staked plants growing in the greenhouse the lesions are invariably located within a foot of the ground, or in other words they are confined to the region where there is opportunity for infested soil to be splashed in watering. The stem appears brown in colour and somewhat constricted over the area covered by the lesion. It also feels spongy due to the destruction of the parenchymatous tissues. If the stem is cut open at this point (Plate I, Fig. 11), only the woody vascular tissues will be found intact.

The variation in the appearance of the disease on the stem may be related to the age of the plant and its rate of growth, and also to environmental conditions. Occasionally the lesions cease to advance before girdling is complete. The plant may then recover, forming cork over the lesion. Young actively growing parts of the stem tend to be more susceptible than older, hardened parts. A pot-bound plant, inoculated by placing a drop of zoospore suspension on each internode and kept in a moist chamber for a few days, will show symptoms of disease only at the uppermost three or four internodes. If soil is piled around the stem of a plant with a basal lesion, the plant may

form adventitious roots above the lesion and survive. On the other hand the lesion may continue up the stem, especially if the tissues are young, and death is merely delayed by the treatment. Under very humid conditions lesions tend to extend further along the stems than they do in a drier atmosphere.

The foliage of the host is affected both directly and indirectly by the disease. In mature plants, usually the first sign of disease that is noticed by the grower is a sudden wilting of the whole plant. This is a secondary effect resulting from the destruction of the stem at a basal lesion. Such wilting does not occur for some time after girdling is complete, so that the foliage of non-staked plants may appear fresh even when the stem can no longer support it. This suggests that the conducting tissue is the last to be destroyed. Wilting of individual leaves occurs also as a result of infection of their petioles. Direct infections are found only on the basal leaves of staked plants, although many natural infections were found on the leaves of non-staked plants growing in infested soil in the field. Such infections kill the tissues, resulting in irregular brownish patches on the leaflets. Leaves, inoculated with drops of zoospore suspension then placed in a moist chamber, show brown lesions within 24 hours. Plate I, Fig 12, shows leaves three days after such an inoculation. Under these conditions the necrotic areas continue to enlarge until the whole leaf is involved.

Fruits in contact with the soil, or near enough to it to become splashed in watering, develop characteristic lesions which spread until the whole fruit is decayed. The surface of the fruit over the diseased area is smooth and not sunken. The lesion has a dark brown centre surrounded by an advancing zone that has a grayish water-soaked appearance. Both green and ripe fruits may be attacked, and the lesion may extend deeply into the tissues (Plate I, Figs 14 and 15). Infected fruits placed in a moist chamber become covered within a day or two with a fluffy mass of mycelium bearing sporangia.

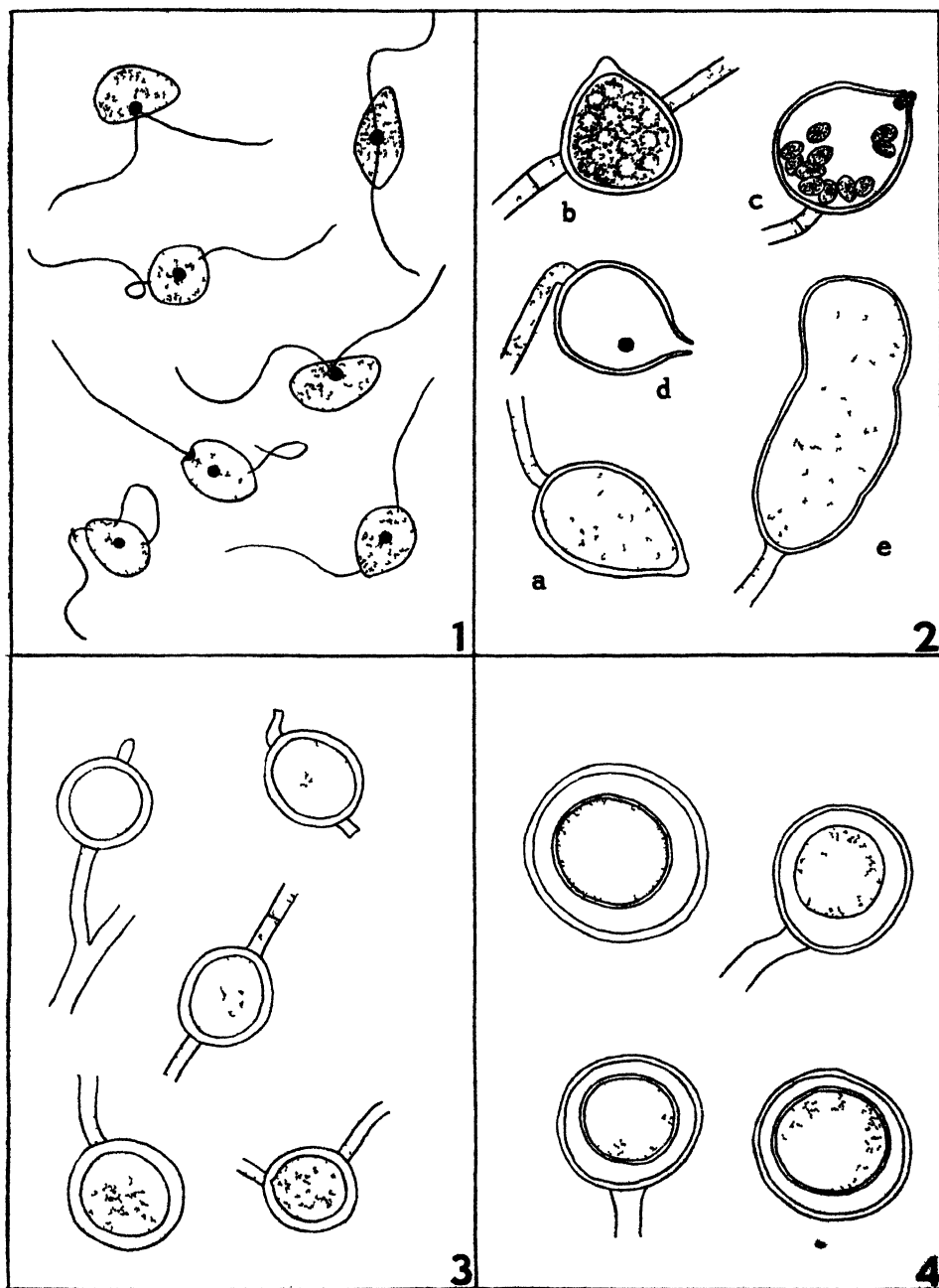
Roots of seedlings grown in infested soil show rotted portions, and mycelium may be observed within the tissues. While it is difficult to demonstrate infection on the roots of mature plants, it is probable that the vigour of all plants growing in infested soil is reduced by such attacks on the root system.

Etiology

The rules of proof of pathogenicity have been applied to the organism causing this disease. It has been found invariably associated with all phases of the disease; it has been repeatedly isolated from diseased tissues and cultured on artificial media; when healthy tissues were inoculated with the fungus from these pure cultures the same symptoms were produced, and the same fungus was recovered on isolation.

A. DESCRIPTION OF THE CAUSAL ORGANISM

Infected fruits or stems kept in a moist chamber soon develop an abundance of white, cottony mycelium. Abundant aerial mycelium is also produced on artificial media, especially oatmeal agar, on which it becomes matted and



FIGS. 1 TO 4. Camera lucida drawings of various organs produced by *P. parasitica* in culture on oatmeal agar.

FIG. 1. Zoospores killed with osmic acid and stained with cotton blue.

FIG. 2. Sporangia; a to d—various stages in formation and liberation of zoospores, e—abnormal vesicular sporangium formed in pea broth.

FIG. 3. Chlamydospores.

FIG. 4. Oospores.

tough in tube cultures. The hyphae range from 3 to 6μ in diameter, are moderately branched, and become sparsely septate with age. Submerged hyphae are irregular in outline and in branching.

Sporangia are produced on the aerial mycelium that forms on the surface of infected fruits or stems under very humid conditions. They are also produced abundantly in culture. Sporangioophores are undifferentiated from sterile hyphae. Sporangia are broadly ovoid to pyriform in shape, each having a very prominent papilla (Fig. 2 and Plate II, Figs. 16 and 17). Both terminal and intercalary sporangia are found, the former often breaking off but without retaining a pedicel. From measurements of 100 sporangia produced on oatmeal agar their length was found to range from 20.3 to 63.8μ , and to average 40.8μ ; their width varied from 14.5 to 49.3μ and averaged 30.0μ ; the mean ratio of length to width was found to be 1.35. The contents of mature sporangia appear fairly homogeneous, but, if they are placed in water, zoospores become differentiated within them and are released within 15 to 20 minutes. Each sporangium produces 20 to 30 zoospores. A few sporangia that had germinated by means of germ tubes were observed, one tube apparently forming for each zoospore that failed to emerge.

At the time of their discharge the zoospores are fusiform in shape, 7.7 to 11.0μ long and 4.4 to 7.2μ wide (Fig. 1 and Plate II, Figs. 18 and 19). Each bears two flagella, differing slightly in length, which are attached at a point on the side of the spore in the vicinity of the nucleus. The zoospores swim about actively for about 30 minutes, then settle down, lose their flagella, and become spherical in shape. In this encysted condition they are approximately 8 to 10μ in diameter. They immediately develop germ tubes, the growth of which is sufficiently rapid to be observed with a microscope. These soon become branched and quite extensive. Their development was observed in Petri dishes on moist cellophane, from which they appeared to derive some nourishment since they produced rather extensive colonies which in a few cases bore new sporangia. Cellophane was used by Haskins (7) for the culture of chytrids.

In cultures one month or more old, spherical chlamydospores with thick brownish walls are found (Fig. 3 and Plate II, Fig. 21). Their diameter was found from 100 measurements to vary from 20.3 to 40.6μ , and to average 31.2μ . They are mostly intercalary and apparently germinate only by germ tubes.

As yet no oospores have appeared in culture on any of the media used. All attempts to induce oospore formation by special treatments failed. When sterile mycelium was transferred from a decoction of canned peas to sterile water (Leonian's (13) method), only sporangia were formed, some of which were abnormally large and vesicular (Fig. 2e). Likewise, only sporangia formed when sterile mycelium was transferred to Petri's mineral solution or to *M*/100 potassium nitrate solution (Tucker's (26) methods). No oospores were formed when local isolants were allowed to grow in contact with the isolant sent from British Columbia. A few oospores were discovered, however,

on the surface of roots of a tomato seedling grown in sterile culture then transferred to a test tube of water into which zoospores had been released. Measurements of 37 oogonia showed a range in diameter from 30.0 to 60.0 μ and a mean diameter of 50.3 μ . The oospores themselves measured 22.5 to 42.5 μ , with a mean diameter of 33.0 μ (Fig. 4 and Plate II, Fig. 20). It was impossible to determine at this stage whether the attachment of the antheridia was amphigynous or paragynous.

B. IDENTIFICATION OF THE CAUSAL ORGANISM

From the above observations it was concluded that the causal organism is *Phytophthora parasitica* Dast. In its morphology it corresponds with that species as originally described by Dastur (5) in 1913 and amended by Ashby (1) in 1928. Because of the large oospores it would be placed in the group *Macrospora* established by Ashby. Tucker (26) states that this species "may be distinguished by its growth on media, papillate sporangia, abundant chlamydospores in culture, amphigynous antheridia and growth on corn meal agar at 35° C." With reference to growth on culture media he further states that ". . . *P. parasitica* usually produces aerial mycelium in profusion. The growth in Petri plates is seldom radiate and appressed . . . , but tufted and irregular with more or less aerial mycelium." The tufted appearance was especially marked in the present study in colonies on corn meal agar. Also according to Tucker "the occurrence of oogonia and oospores in culture is uncertain. Isolations seldom produce them very promptly; they may appear only after several months or, frequently, not at all." If Leonian's (13) key for identification were followed, this fungus would be identified as *P. palmivora* Butl., since he includes *P. parasitica* with other species in this one species.

Cultural Studies

A. MEDIA

This species of *Phytophthora* grows well on a variety of culture media, including potato dextrose agar, malt agar, and corn meal agar. Of the media tried, unfiltered oatmeal agar supported the most rapid and luxuriant growth of mycelium with abundant sporangia. A suitable liquid culture medium was made up according to the following formula: Bacto peptone, 2.0 gm.; dihydrogen potassium phosphate, 0.5 gm.; magnesium sulphate, 0.5 gm.; succinic acid, 0.2 gm.; dextrose, 5.0 gm.; distilled water, 1000 cc. This is a modification of Leonian's (13) medium, with Bacto peptone substituted for proteose peptone. The fungus does not fruit on this medium unless the colonies reach the surface. Its growth was accelerated slightly by the addition of a trace of Vitamin B₁ in the form of thiamin hydrochloride. Growth was greatly stimulated when the roots of tomato seedlings were added to the medium before autoclaving it. The root of a week-old seedling added to 100 cc. of medium brought about a marked increase in growth. It is not known whether the roots provided a nutrient substance or a growth promoting substance.

B. EFFECT OF TEMPERATURE ON GROWTH RATE

On Oatmeal Agar

The effect of temperature on the growth rate of *P. parasitica* was first studied using unfiltered oatmeal agar as the medium. The following temperature range was obtained by using refrigerators, Wisconsin temperature tanks, and incubator ovens: 2°, 5°, 8°, 12°, 16°, 21°, 30°, 32.5°, 37.5°, and 40° C. Three Petri dish cultures were grown at each temperature, and dishes of water were kept in the ovens at 35° to 40° to check the drying of the medium. Uniform disks of inoculum were cut from the margin of a vigorously growing colony on oatmeal agar in a Petri plate by means of a platinum "biscuit cutter". The individual disks were then transferred to the centres of the plates. The outlines of the colonies were marked with India ink on the bottoms of the plates at approximately 12-hour intervals until the colonies had reached the margins of the dishes.

No growth resulted at 2°, 5°, and 8°, or at 35°, 37.5°, and 40° C. After 146 hours one plate from each of these temperatures was brought to 26° and kept there until the end of the experiment. The cultures from the lower temperature group survived and developed, but those from the higher temperatures were dead. The growth curve obtained for the cultures at the remaining temperatures show the minimum temperature for growth on this medium to be about 12° and the maximum temperature about 32.5° C. The greatest amount of growth under these conditions is made over the range from 26° to 32.5° C.

On Potato Dextrose Agar

The experiment was repeated over the same temperature range using as the medium potato dextrose agar. Unfortunately the cultures at the temperatures from 12° to 21° were destroyed through accidental flooding in the temperature tanks. The fungus makes much slower growth on this medium and the colonies are more submerged and irregular in outline. The best growth was made over the same range as before with the largest colonies at 26° C.

On Corn Meal Agar

The experiment was repeated at a later date using filtered corn meal agar as the medium. The temperatures used this time were 8°, 12°, 15°, 18°, 25°, 30°, 32°, 35°, 37.5°, and 40° C. After 168 hours at these temperatures all cultures were brought to room temperature (approximately 20° C.) and kept there for 92 hours longer.

The diameters of the colonies produced by the fungus on these three media at the various temperatures are shown in Fig. 5. The cardinal points obtained for corn meal agar do not closely agree with those found for growth on the other media. The minimum temperature was the same, although much better growth was made at 12° on the corn meal agar. Fairly good growth was obtained at 35° where none resulted before. Even at 37.5° the

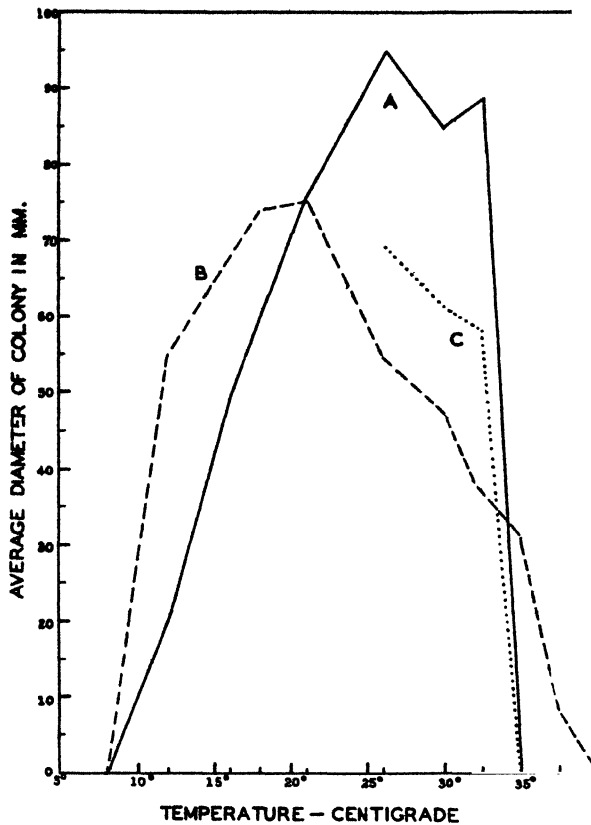


FIG. 5. Effect of temperature on the growth rate of *Phytophthora parasitica* on different media. Diameters of colonies produced at the various temperatures by the end of the test periods.

A. Oatmeal agar, 88 hours.

B. Corn meal agar, 168 hours.

C. Potato dextrose agar, 260 hours.

fungus developed for a few days before it was killed. The temperature range that is optimum for growth on corn meal agar (18° to 21°) is lower than that for growth on oatmeal and potato dextrose agars (26° to 32°). The variation found here in the cardinal points on different media illustrates the necessity for caution in interpreting the effect of the temperature factor on disease incidence in terms of its effect on the vegetative development in culture.

The type of growth produced on corn meal agar changed between 21° and 25° . At 21° and below aerial mycelium was abundant, erect strands of hyphae reaching the lids of the dishes. At 25° and above growth was closer to the surface of the medium and more compact. The margins of the colonies also became more irregular with increasing temperature. The difference in growth habit at this point may be due, in part at least, to the difference in the relative humidity of the atmosphere, since the lower series was kept in tanks with the temperature controlled by flowing water, while the higher series was kept in incubator ovens.

The reactions of the cultures when returned to room temperature are interesting. The cultures from 37.5° and 40° did not grow at all at this temperature, while the cultures that had been kept at 8° developed rapidly. All the other cultures continued to grow, although not all at the same rate. Those that had previously grown fastest at 18° to 25° and covered the greatest area now spread at a slower rate than those (from higher or lower temperatures) that had covered less area. This shows that temperature and not drying of the medium was responsible for the slower growth rates at temperatures above the optimum under these conditions.

C. EFFECT OF ATMOSPHERIC HUMIDITY ON THE GROWTH OF THE FUNGUS IN CULTURE

The effect of the relative humidity of the atmosphere on the growth of *P. parasitica* in culture was studied, following a procedure outlined by Hopp (10). The relative humidity within the confined space of Petri dishes was controlled by using different concentrations of sulphuric acid. Eight cubic centimetres of oatmeal agar were poured into each of 24 75-mm. Petri dishes. Each plate was inoculated as before by inoculum-disk method, then placed within a sterile 100 cc. Petri dish. The lid of the smaller dish was removed, and 15 cc. of sulphuric acid of the required concentration was poured into the outer dish. The concentration of acid required to maintain a given relative humidity in a closed atmosphere over it was found from Hopp's graph. Solutions were prepared that produced atmospheres with initial relative humidities ranging from 0 to 100% with 10% intervals. Plates were prepared in duplicate, and one pair with no solution was used as a control. All plates were incubated at 30° C. for 66 hours, then the diameter of the colony in each was measured.

The size of colony produced in each atmosphere is shown in Table I. No aerial growth was produced at any relative humidity lower than 80%. Below that humidity submerged colonies were formed inconsistently, ranging in size from 10 to 35 mm. These evidently represented the amount of growth possible on the amount of moisture supplied by the medium. The agar at the lower humidities soon dried and cracked. Above 80% relative humidity the colony size increased with increasing humidity to a diameter of 61 mm. at saturation. The check plates produced colonies of a size between those formed at 90 and 100% saturation. Moisture, apparently supplied by the freshly poured agar, condensed on the lids of these dishes, and this is probably the condition in Petri dish cultures during the first few days. The relative humidity later will depend upon the temperature that determines the rate of evaporation. Atmospheric humidity was probably one factor that determined the rate and amount of aerial growth found at different temperatures in the preceding experiment. This factor may also influence the development of the fungus in nature, and explain in part the restriction of lesions to the region of the stem near the ground level.

TABLE I
RELATION BETWEEN ATMOSPHERIC HUMIDITY AND THE GROWTH
OF *P. parasitica* IN CULTURE

Conc'n of H_2SO_4 , %	Relative humidity, %	Diameter of colony, mm.*		
		Plate 1	Plate 2	Average
95.0	0	35.0	—	35.0
67.0	10	22.0	24.0	23.0
59.0	20	27.0	23.0	25.0
53.5	30	17.0	10.0	13.5
49.0	40	18.0	—	18.0
43.0	50	15.0	—	15.0
38.0	60	33.0	—	33.0
34.0	70	35.0	x	35.0
27.5	80	37.0	41.0	39.0
19.5	90	46.0	49.0	47.5
Water	100	63.0	59.0	61.0
—	Check	55.0	62.0	58.5

* After 66 hours at 30° C.

x = Contamination.

D. EFFECT OF ACIDITY OF MEDIUM ON GROWTH RATE

In the study of the relation between the pH of the medium and the growth rate of the fungus in culture the liquid medium previously described was used. A preliminary series of solutions was made up with this to determine the amount of 1/10 *N* hydrochloric acid or 1/10 *N* sodium hydroxide required to adjust them to desired pH concentrations. In the solutions actually used for cultures, 50 cc. of water with the nutrient salts were put into each 125 cc. Erlenmeyer flask, while the acid or base was run into a test tube then diluted to 25 cc. with distilled water. Flasks and tubes were plugged and autoclaved separately, then the contents of the tubes were added to the flasks immediately before inoculation. The inoculum disk method of inoculation was used as before, care being taken that the disks settled to the bottom of the flasks so that all growth was submerged. Four flasks were prepared for each of the pH concentrations tested. After inoculation they were kept in an incubator for seven days. The four corresponding cultures were then filtered through a previously weighed filter paper; this was air-dried for 24 hours, then weighed again. The difference between these weights was taken as the dry weight of the mycelium produced. The pH of the filtrate was determined electrometrically. The experiment was repeated over approximately the same pH range.

The original and final pH of the cultures and the dry weights of the mycelium produced in them are shown in Table II. The fungus exhibited a wide range of tolerance with respect to the reaction of its substrate. Growth was possible in the acid range at pH 3.5 and in the alkaline range at pH 9.5. Maximum growth under the conditions of the experiment was attained at pH 5.0. The reaction of the medium was not shifted appreciably except in the alkaline

TABLE II

EFFECT OF pH OF MEDIUM ON GROWTH RATE OF *P. parasitica*
IN LIQUID CULTURES

Original pH	Final pH		Dry weight of mycelium, mg.	
	1st test	2nd test	1st test	2nd test
3.0	3.02	—	0.0	—
3.5	3.48	3.55	60.0	50.2
4.0	3.75	3.80	66.0	57.7
5.0	4.64	4.60	124.3	147.8
6.0	5.68	5.48	97.4	128.9
7.0	6.70	7.00	112.6	77.3
8.0	7.85	8.10	76.2	69.2
9.0	8.12	8.25	83.4	52.7
9.5	—	8.35	—	48.9
10.0	8.70	8.70	Trace	0.0

range above pH 8.0. This is due to the instability of the medium and is not caused by the growth of the fungus, since the shift occurred in the cultures started at pH 10.0 where no growth ensued. A slight precipitation was observed in those cultures whose reaction shifted markedly. The slight shifts in the other cultures was usually in the direction of increased acidity.

Epidemiology

A. ATMOSPHERIC ENVIRONMENT

Temperature and Relative Humidity

The intensity of the disease caused by this pathogen is influenced markedly by atmospheric conditions. While it was not feasible to carry out experiments under controlled conditions of atmospheric temperature and humidity, observations were made in the course of experiments involving thousands of seedlings grown under varying conditions. From these and from observations made whenever natural epidemics occurred in commercial greenhouses it was concluded that maximum disease incidence was correlated with high air temperatures and high relative humidity. The rate of damping-off of seedlings was greatest on hot, humid days. Natural infection of mature plants occurred only in the months of June, July, August, and September, even where the soil was heavily infested. Temperature and humidity appear, therefore, to be the limiting factors which, directly or indirectly, restrict the disease almost exclusively to plants grown under glass.

B. SOIL ENVIRONMENT

Temperature and Moisture

The influence of soil temperature and soil moisture on disease incidence in tomato seedlings was investigated by using the Wisconsin soil temperature tanks in the University of Toronto greenhouses. The air temperature was kept at about 21° C. and the relative humidity varied from about 85 to 90%.

The soil used was a mixture of one-third each of loam, sand, and leaf mould. This was steam sterilized, then infested by adding cultures of *Phytophthora parasitica* growing on a corn meal and sand mixture. Sixteen 500 cc. Erlenmeyer flasks of inoculum were used for about five cubic feet of soil. After thorough mixing, one portion of the soil was adjusted to each of three moisture levels. The first lot was left too dry for good development of tomato seedlings; the second was given a moisture content that was approximately optimum for the host; the third was made too wet for the best development of the host. These three moisture levels will be referred to as "low", "optimum", and "high". Their moisture contents, determined at the end of the experiment from the top two inches of soil, were found to be respectively 34.0, 49.0, and 71.5% of the moisture-holding capacity of this soil.

Each of the soil cans was prepared for planting in the following way. First a 3-in. layer of sterilized sand was put in the bottom for drainage. On top of this an inverted 2-in. flower pot was placed with a glass tube leading from it up to an inch or more above the infested soil which was filled in next. Water was added as required through the glass tube and the pot acted as a reservoir to permit uniform distribution of moisture through the soil and to avoid saturation of the surface layer. The weight of each can was determined at the beginning of the experiment, and the required moisture content was maintained by adding water to bring each can back to its original weight. The need for additional water varied with the temperature. Cans at the higher temperatures were checked every second day, while those at the lower temperatures were checked once a week.

Duplicate cans were prepared in this way at each of the three moisture levels for each of five temperature tanks. The tanks were maintained at five different temperatures: 12°, 17°, 22°, 27°, and 32° C. In each can 150 tomato seeds of the variety Vetomold were planted at a uniform depth. The soil was covered with a layer of non-absorbent cotton to conserve moisture until the seedlings emerged.

Daily records of the number of seedlings damped-off were taken until 36 days after the date of planting. The survivors were then counted and the daily losses totalled. The difference between the total number of seedlings that emerged and the number of seeds that were planted was taken as the amount of pre-emergence blight. The total killing (pre-emergence plus post-emergence) was then expressed as a percentage of the number of seeds planted.

A check series was planted at the same time to ascertain the effect of soil temperature and soil moisture on the germination of tomato seeds in the absence of the pathogen. One check can filled with sterilized soil with optimum moisture content was kept in each temperature tank. In addition, one can of sterilized soil at each moisture level was kept at room temperature (about 21° C.) for the duration of the experiment.

The results for the control series, which are presented in Table III, were not used in computing the amounts of pre-emergence killing in the cans of infested soil. The 26% germination obtained at 12° C. is evidently exceptional,

TABLE III

EFFECT OF VARIOUS TEMPERATURES AND MOISTURE CONTENTS OF STERILIZED SOIL ON THE GERMINATION OF TOMATO SEEDS

Temperature, °C.	Moisture	No. of seeds germinated	Germination, %
12	Optimum	39	26.0
17	Optimum	128	85.3
22	Optimum	143	95.3
27	Optimum	150	100.0
32	Optimum	118	78.6
±21	Low	140	93.3
±21	Optimum	146	97.3
±21	High	134	89.3

since 85% was obtained in the corresponding cans of infested soil. The same discrepancy was found, to a lesser degree, in the other cases. A larger number of replicates at each combination of temperature and moisture would have to be used to give a reliable basis for comparison, but the equipment available would not accommodate them. While assuming 100% germination in the analysis of the results may alter slightly the absolute amount of total killing and the relation between pre- and postemergence killing, this would not distort appreciably the effect of any soil temperature or soil moisture on disease incidence.

TABLE IV

EFFECT OF SOIL MOISTURE AND SOIL TEMPERATURE ON THE AMOUNT OF DAMPING-OFF OF SEEDLINGS DUE TO *P. parasitica*

Soil temperature, °C.	Soil moisture	Survivors, 36 days	Emergence		Damping-off			
			Total	%	Pre-emergence	Post-emergence	Total	%
12	Low	243	245	81.7	55	2	57	19.0
	Opt.	251	255	85.0	45	4	49	16.3
	High	196	234	78.0	66	38	104	34.7
17	Low	152	269	89.7	31	117	148	49.3
	Opt.	113	254	84.7	46	141	187	62.3
	High	27	225	75.0	75	198	273	91.0
22	Low	137	255	85.0	45	118	163	54.3
	Opt.	104	217	72.3	83	113	196	65.3
	High	21	176	58.7	124	155	279	93.0
27	Low	174	261	87.0	39	87	126	42.0
	Opt.	131	211	70.3	89	80	169	56.3
	High	72	300	100.0	0	228	228	76.0
32	Low	205	272	90.7	28	67	95	31.7
	Opt.	146	265	88.3	35	119	154	51.3
	High	79	256	85.3	44	177	221	73.7

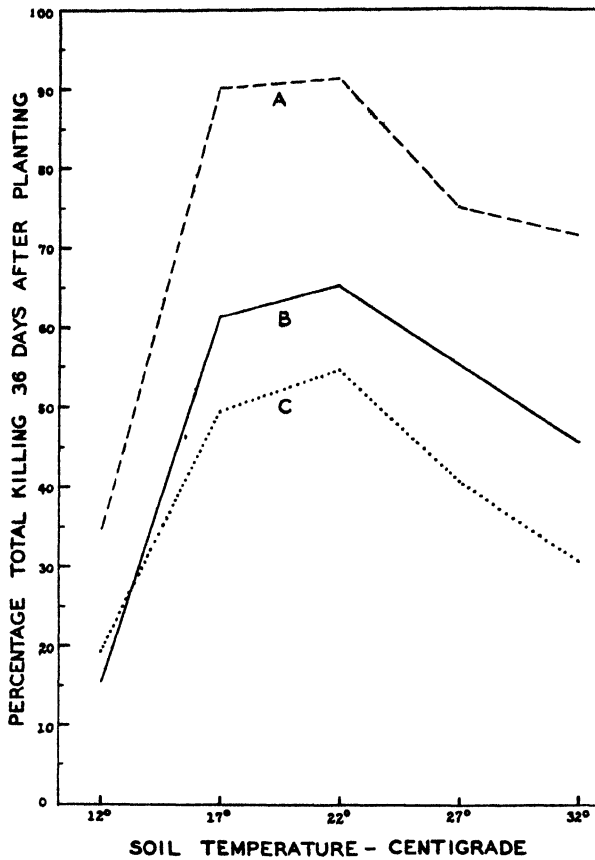


FIG. 6. Effect of soil temperature and soil moisture on the total killing of tomato seedlings by *Phytophthora parasitica* in 36 days after planting.

- A. High moisture.
- B. Optimum moisture.
- C. Low moisture.

The final results of the experiment are shown in Table IV and Fig. 6. The relation between disease incidence and soil moisture is apparent, the amount of disease varying directly with the moisture content of the soil. A broad temperature range from about 15° to 27° C. is favourable for disease, while maximum disease occurs at 22° C. The steepest decline in disease incidence occurs between 17° and 12°. The lack of separation of the curves for low and optimum moisture at 12° may be attributed to the fact that condensation of atmospheric moisture on the sides of the cans and on the soil surface at this temperature made it impossible to keep the low moisture cans at their original moisture content.

On comparing the effect of temperature on disease with its effect on the growth of the causal organism in culture, it is found that the temperature range 15° to 27° which is most favourable for disease also permits good vegetative development of the fungus in culture. Thus the temperature factor

apparently influences the degree of disease incidence largely through its direct effect on the pathogen. That temperature may also favour disease by predisposing the host to attack is suggested by the fact that maximum disease occurs at a point below 27° C. which is the most favourable temperature for the development of the host. At temperatures below the optimum range for disease, the host takes longer to emerge from the soil and pre-emergence blight is greater, in spite of the fact that the pathogen is less active, due to the longer exposure to attack at this stage. The decrease in postemergence killing at low temperatures is apparently due to a depressing effect of the temperature on the development of the pathogen.

C. FACTORS INFLUENCING THE ESTABLISHMENT AND THE ACTIVITY OF THE PATHOGEN IN THE SOIL

ESTABLISHMENT IN THE SOIL

Effect of Soil Type

In the following experiment the ease of establishment of the pathogen in two different types of soil was compared. The soils used were an acid, marsh soil, or "muck", and a neutral garden loam. The muck soil was taken from a swamp that is flooded most of the year. Its pH was found to be 6.6, while the loam had a pH of 7.0. One lot of each soil was steam sterilized but this treatment did not change the reaction. One flat was then filled with each of the soil lots—sterile and non-sterile muck, and sterile and non-sterile loam. Each flat was then infested by adding one culture of *P. parasitica* growing on corn meal and sand in a 300 cc. Erlenmeyer flask. After thoroughly mixing the inoculum into it, the soil was left for 10 days before planting. Then 500 seeds were planted at a uniform depth in each flat, as well as in a series of non-infested check flats of each soil lot.

The check series showed no significant difference in the amount of germination in the loam and muck, either sterile or non-sterile. Since the germination was uniformly high in the checks, pre-emergence killing was estimated on the basis of the number of seeds planted. The results of this experiment are recorded in Table V. The pathogen was evidently more successful in estab-

TABLE V

RELATIVE AMOUNTS OF DISEASE IN INFESTED STERILE AND NON-STERILE LOAM AND MARSH SOIL

Soil type	Survivors, 30 days	Emergence		Damping-off			
		Total	%	Pre-emergence	Post-emergence	Total	%
Non-sterile muck	203	370	74.0	130	167	297	59.4
Sterilized muck	198	361	72.2	139	163	302	60.4
Non-sterile loam	174	450	90.0	50	276	326	65.2
Sterile loam	29	354	70.8	146	325	471	94.2

lishing itself in some of the soil lots than in others, according to the trends shown in disease incidence. The amount of total killing was approximately the same in the infested sterile and the non-sterile marsh soil, but it was much greater in the sterile loam than in the non-sterile loam. The amount of pre-emergence killing in non-sterile loam was roughly only one-third of that in the other three lots, though here the postemergence killing was much higher than in the muck soils.

The two soil types differed, presumably, (i) in their microfloral population, as well as (ii) in acidity, and (iii) in physical condition. The differences in ease of establishment of the pathogen in them might, therefore, be attributed to one or more of these features. That the microfloral populations were a factor is shown by a comparison of the disease trends in the sterile and the non-sterile series. The difference in disease incidence in sterile and non-sterile loam is very striking. Pre-emergence killing was 50 in non-sterile loam and 146 in sterile loam, while postemergence killing was 276 and 325, respectively. Thus some factor, which is present in non-sterile loam but not in sterile loam, suppresses temporarily the aggressiveness of the pathogen. This factor is most likely the competitive effect between the pathogen and the other soil organisms present in the non-sterile loam. This evidently did not affect the establishment of the pathogen in non-sterile muck, since the amounts of both pre- and postemergence killing there were practically identical with the amounts in the sterilized muck. The pathogen apparently found the predominant members of the microfloral population of this sample of marsh soil compatible, whereas it had more difficulty in establishing itself in the presence of the microflora of the loam. Whether this difference was due to the specific effect of one organism present in the one soil and not in the other, or whether it is due to a difference in the resultant of the dominant forms in the two cases could only be shown by a study of the soil populations by isolation methods. The slight difference in acidity between the two series was evidently not a factor influencing establishment, since no correlation was found between pH and disease. The physical condition of the soil was apparently a factor, since disease incidence was much higher in the sterile loam than in the sterile muck when the biological factor, except for saprophytic forms that come in after sterilization, is ruled out. This physical factor seems to have more effect on the postemergence killing than on the pre-emergence, since twice as much postemergence killing occurred in the sterile loam as in the sterile muck whereas the amounts of pre-emergence killing were roughly the same. One physical character of the soils that could conceivably influence disease incidence in this way is the water-holding capacity. The marsh soil absorbed water very readily and remained porous, while the loam was easily saturated and remained soggy for some time after watering. The pathogen would likely have an equal chance in the two cases of attacking seedlings before they emerged, but if it depended on free water to release zoospores for attack on aerial portions of the seedlings, it would be more successful in the loam.

Effect of Mixing Infested Soil with Various Proportions of Sterile and Non-sterile Soil

In order to study further the competitive effects influencing the establishment of the pathogen in the soil, the following experiment was carried out. Using the infested soil which had been inoculated four months previously and used subsequently in the soil temperature experiment, a dilution series was made up. In the first half of the series the infested soil was mixed with various amounts of freshly mixed greenhouse soil composed of one-third loam, one-third leaf mould, and one-third sand. In the other half of the series the infested soil was mixed with the same amounts of steam sterilized soil of the same composition. One flat was then made up with each of the following proportions of infested and non-infested soil.

Flat No.	Proportions		Flat No.	Proportions	
	Infested	Non-sterile		Infested	Sterile
1	All	None	8	7/8	1/8
2	7/8	1/8	9	3/4	1/4
3	3/4	1/4	10	1/2	1/2
4	1/2	1/2	11	1/4	3/4
5	1/4	3/4	12	1/8	7/8
6	1/8	7/8	13	None	All (check)
7	None	All (check)			

In the first test, all flats were planted May 22 with 500 seeds each and covered to a uniform depth with the appropriate soil mixture. Daily records of the number of seedlings killed were kept for 30 days after planting. The survivors were then removed and counted, and the amount of pre-emergence killing was computed by subtracting the total number of seedlings that emerged in each flat from the number that germinated in the corresponding sterile or non-sterile check. The total killing was also expressed as a percentage of the germination in the appropriate check.

The second planting was made on June 24 in the same flats. The soil was loosened by stirring, then an equal part was removed from each and used for covering the seeds after planting. Records were kept for another 30 days, then the results were computed as before.

The results obtained from both plantings are shown in Table VI. The amount of total killing in the flats in the first planting varied with the relative amount of infested soil in the various mixtures. Thus maximum killing resulted in the flat of undiluted infested soil (Flat 1), and the disease incidence in the other corresponding pairs decreased with decreasing amounts of infested soil. Flat 4 was abnormal due to accidental flooding two days after planting. That flat remained much wetter than the others for a week or more though not watered further. Pre-emergence killing was exceptionally high in this case. Postemergence killing was also high for a few days but fell to a level

TABLE VI

AMOUNTS OF DISEASE CAUSED BY *Phytophthora parasitica* IN SEEDLINGS PLANTED IN INFESTED SOIL MIXED WITH VARIOUS PROPORTIONS OF STERILE AND NON-STERILE SOIL

Flat No.	Composition of soil	First planting						Second planting					
		Survivors, 30 days	Total emergence	Damping-off				Survivors, 30 days	Total emergence	Damping-off			
				Pre-emergence	Post-emergence	Total	%*			Pre-emergence	Post-emergence	Total	%*
1	All infested	21	418	52	397	447	95.5	129	431	46	302	348	72.9
2	7/8 infested 1/8 non-sterile	17	425	25	408	433	96.2	66	422	23	356	379	85.1
3	3/4 infested 1/4 non-sterile	33	423	27	390	417	92.7	143	418	27	275	302	67.8
4	1/2 infested 1/2 non-sterile	167	336	114	169	283	62.9	121	435	10	314	324	72.8
5	1/4 infested 3/4 non-sterile	83	436	14	353	367	81.6	110	419	26	309	335	75.3
6	1/8 infested 7/8 non-sterile	125	448	2	323	325	72.0	166	385	60	219	279	62.6
7	All non-sterile (check)	448	450	0	2	2	0.4	445	445	0	0	0	0.0
8	7/8 infested 1/8 sterile	25	433	35	408	443	94.6	62	421	56	359	415	87.0
9	3/4 infested 1/4 sterile	29	456	12	427	439	93.8	47	402	75	355	430	90.1
10	1/2 infested 1/2 sterile	27	452	16	425	441	94.2	61	358	119	297	416	87.2
11	1/4 infested 3/4 sterile	59	475	—7	416	409	87.3	116	378	99	262	361	75.6
12	1/8 infested 7/8 sterile	113	468	0	355	355	75.8	148	376	101	228	329	68.9
13	All sterile (check)	468	468	0	0	0	0.0	477	477	0	0	0	0.0

* Percentage of germination in corresponding check.

lower than that found in any of the other flats. The seedlings in this flat remained dwarfed and yellowish in colour as a result of the excess moisture. This hardened condition of the plants may account for the early decline in disease incidence there. When corresponding pairs of the normal flats are compared, it is found that the amount of total killing is invariably greater in the case of the flat diluted with sterile soil than in that diluted with non-sterile. This difference became more apparent as time went on, suggesting that the pathogen was becoming established with greater success in the sterile soil than in the non-sterile.

The results of the second planting show a further reduction in disease in the non-sterile as compared with the sterile mixtures. This confirms the tendency shown in the first planting for the pathogen to be suppressed in its aggressiveness, apparently by some biological factor present in the non-sterile soil. Whereas the results in the sterile series appears like a dilution effect with increasing admixtures of sterile soil, the non-sterile series exhibits a definite suppressive effect on the ability of the pathogen to cause disease. Both pre-emergence and postemergence killing were greater in the sterile mixtures than in the non-sterile mixtures. In the first planting the amount of pre-emergence blight was roughly the same in all normal cases. While in the second planting the non-sterile mixtures still showed the same amount, in the sterile mixtures it was doubled. Apparently the pathogen had become better established there, probably due to the initial absence of competition.

There was considerably less killing during the second test in the flat of undiluted infested soil (Flat 1). The reason for this is not clear. It suggests the staling effect found in old cultures of fungi. It might be the result of competition with saprophytic micro-organisms coming into the once sterile soil. It is also possible that exhaustion of nutrient salts, especially nitrates, resulting from the repeated plantings of tomato seedlings in the soil, increased the resistance of the host to attack. This would be overcome in the other flats which received a portion of fresh soil.

Decline in Degree of Soil Infestation with Time

The degree of infestation was determined for the third time for the most dilute mixtures of infested and non-infested soil—i.e., the 1/8 : 7/8 mixtures in Flats 6 and 12. These were replanted as before on August 13 and the course of the disease was followed for another 30-day period.

The data for these flats in all three trials are brought together in Table VII. From these it is evident that the disease rate falls off appreciably as time

TABLE VII

DECLINE IN THE DEGREE OF INFESTATION OF SOIL BY *P. parasitica* AS SHOWN BY THE AMOUNTS OF KILLING OF TOMATO SEEDLINGS PLANTED IN IT

Composition of soil	Date of planting	Survivors, 30 days	Emergence		Damping-off			
			Total	%	Pre-emergence	Post-emergence	Total	%*
No. 6 1/8 infested, 7/8 non-sterile	May 22	125	448	89.6	2	323	325	72.0
	June 24	166	385	77.0	60	219	279	62.6
	Aug. 13	264	383	76.6	62	119	181	40.7
No. 12 1/8 infested, 7/8 sterile	May 22	113	468	93.6	0	355	355	75.8
	June 24	148	376	75.2	101	228	329	68.9
	Aug. 13	257	399	79.8	78	142	220	46.1

* Percentage of germination in corresponding check.

goes on. In every case the mixture with sterile soil shows more disease than the mixture with non-sterile soil. The differences are slight, it is true, but they are consistent. The amount of pre-emergence blight in both cases was practically nil in the first trial immediately after mixing the soils, but increased in the later tests, especially in the sterile mixture. This again suggests that the pathogen had not become well established in the soil in the first trial, and that it became more generally distributed, according to the later trials, in the sterile than in the non-sterile mixture.

Field Plot Experiments

The establishment of *P. parasitica* in natural soil was also studied under field conditions. A series of plots were inoculated by adding corn meal and sand cultures of the pathogen. The soil in half of these plots had been previously removed, steam sterilized and replaced to temporarily remove the biological factor. The activity of the parasite was shown by observing the trends in disease incidence in tomato seedlings over two 30-day test periods. The field experiments confirmed the greenhouse tests in demonstrating a retarding factor that is present in non-sterile soils but not present in sterilized soils. Competition with the fungal and bacterial population of non-sterile soils presumably accounts for such an effect. Other organisms may compete for the substrate required by the pathogen, or they may release into the soil by-products that retard its development. No attempt was made to isolate organisms with such antibiotic tendencies.

THE INVASION OF NON-INFESTED SOILS BY THE PATHOGEN

Spread from a Single Centre of Infection

The successful infestation of soil from a single centre of infection originating from a minute amount of inoculum was demonstrated by a simple experiment. Twenty apparently healthy tomato seedlings that had been growing for 30 days in infested soil were transplanted into 5-in. pots, 10 of which contained sterilized greenhouse soil, the other 10 non-sterile soil. As much of the original soil as possible was shaken from the roots before replanting. Two weeks later, four pots were selected from each lot and the plants, which had been killed by that time, were carefully pulled out without disturbing the soil. In each pot 16 seeds were next inserted into the soil, by means of forceps, in the arrangement shown in Fig. 7—i.e., two in the position of the original plant, four in the inner ring about this point, and 10 in the outer ring. For three weeks after their planting the dead seedlings were removed daily and their position was noted. Those surviving were left for a further three weeks before a final count was made.

Most of the seeds planted in the exact position of the original plant did not germinate, and the few seedlings that did come up at that point were soon killed. Seedlings in the other rings were killed progressively outwards from the centre. Fig. 7 represents a typical pot from each lot. Three weeks after planting the situation was as follows: in non-sterile soil—36 alive, 15 damped-

off; in sterile soil—32 alive, 21 damped-off. Three weeks later only two seedlings were alive in the non-sterile soil, while six survived in the sterile. The number of individuals used was small but the experiment does indicate the ability of the pathogen to establish itself and spread in either sterile or non-sterile soil to a degree that makes it a potentially destructive contaminant in greenhouse soil, whether introduced en masse in a soil lot or in minute quantities with young plants or on tools or workers' boots.

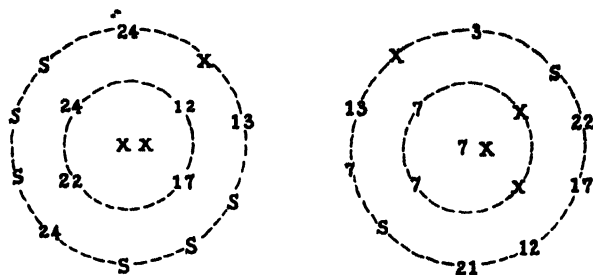


FIG. 7. Diagram showing the spread of the disease from centres of infection; left: non-sterile soil, right: sterile soil. Numerals mark position of dead seedlings and represent number of days between planting and killing.

x:— seedling failed to emerge.

s:— living seedling.

It is interesting to note the behaviour of the original plants in these pots. Although they appeared healthy when transplanted into the pots, within a week every plant showed definite symptoms of infection, and by the end of two weeks all but two had succumbed. One of these was in the sterile soil, the other in the non-sterile. If they had been left undisturbed in the original infested soil very few of them would have been killed at that stage. Thus transplanting appeared either to weaken the resistance of the host to fresh infection by the pathogen or to stimulate the development of incipient or abortive infection.

Linear Spread in Flats of Soil

Experiment I

To study further the relative rates of invasion of sterile and non-sterile soils by *P. parasitica*, the following experiment was devised. A very thin partition of wood was placed across the centre of each of two flats. One-half of each flat was then filled with soil that had been infested four months previously and used in subsequent experiments. The other half of the first flat (A) was then filled with a non-sterile mixture of loam, leaf mould, and sand. The other half of the other flat (B) was filled with the same soil mixture after it had been steam sterilized. Ten rows, two inches apart, were marked across each flat by means of a wooden marker, so that five rows were across each type of soil. Fifty tomato seeds were planted in each row on June 4 and covered with as little disturbance of the soil as possible. The partitions were then removed and the infested and non-infested soils came in contact with one another. Records of the number of seedlings killed each day were kept for 30 days. At the end of that time the survivors were counted

and the number in each row that failed to emerge was determined. The surviving seedlings were carefully removed, fresh rows were marked in the same places as before, and a second planting was made at once (July 4) without disturbing the soil. Records of damping-off were taken for a second 30-day period.

The advance of the pathogen into the non-infested soils may be traced by following the progress of the disease as recorded in Table VIII. The results at the end of each period are presented in the form of a histogram in Fig. 8. During the first test the spread of the pathogen into the non-sterile

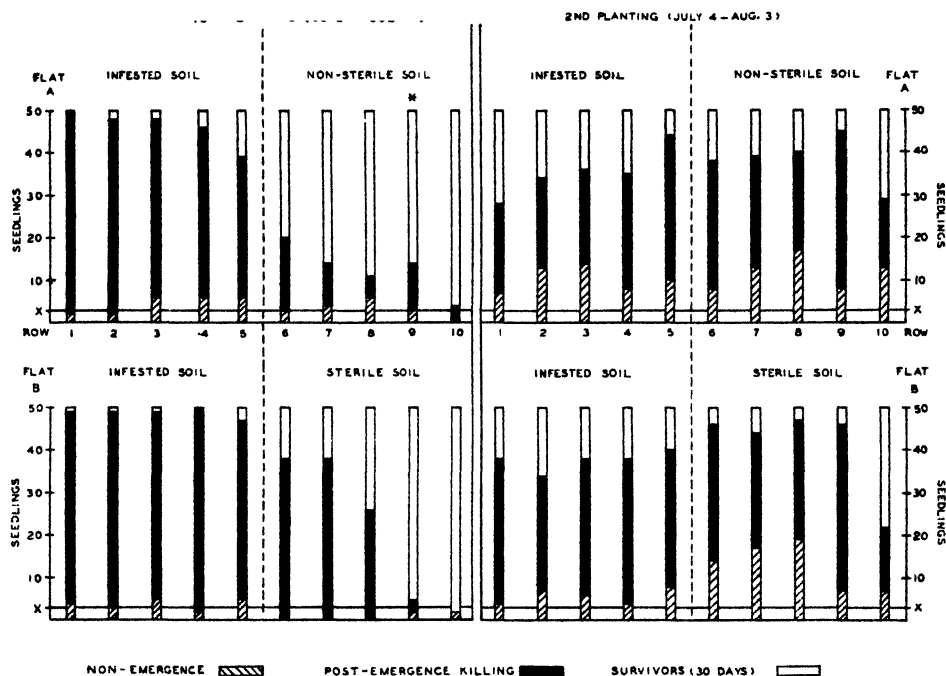


FIG. 8. Histogram showing the advance made by *P. parasitica* from infested soil into sterile and non-sterile soils by the end of 30 days and 60 days, as indicated by the numbers of seedlings killed in each row. Horizontal line at *x* indicates the average amount of non-emergence obtained for this seed lot when planted in non-infested soil.

* Surface contamination.

soil in Flat A was slow and irregular. A spot infection that occurred in Row 9 was apparently the result of surface contamination, since the rows between it and the infested soil escaped disease until later. The advance of the pathogen into the sterile soil in Flat B was more rapid and uniform, progressing row by row, and reaching Row 9 by the 30th day.

During the second trial the pathogen became well established throughout the non-sterile soil in Flat A. It seemed to spread from two centres, from the line of contact with the infested soil and from the contaminated spot mentioned previously. The sterile soil in Flat B became heavily and uniformly infested. About 90% mortality resulted in all rows there except Row 10

TABLE VIII

INVASION OF STERILE AND NON-STERILE SOILS OF *P. parasitica* AS SHOWN BY THE PROGRESSIVE DAMPING-OFF OF SEEDLINGS
DAILY LOSSES UP TO 30 DAYS FROM PLANTING

Soil	Row	Number of days from planting																											
		7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				
Flat A—Non-sterile	Infested	—	—	0	0	0	2	—	0	1	0	0	1	2	—	6	8	17	7	1	0	—	—	3	0	0	0	0	0
		—	3	13	3	1	—	—	1	0	0	0	1	—	8	3	6	5	1	0	—	—	—	1	0	0	0	0	0
		—	1	15	2	3	—	—	0	0	1	0	0	1	—	9	1	5	3	0	1	—	—	—	0	0	0	0	0
		—	1	5	3	6	—	—	1	2	1	0	0	5	—	12	4	0	0	0	—	—	—	0	0	0	0	0	0
		—	0	0	1	1	—	—	4	1	2	0	2	8	—	15	0	0	2	1	0	—	—	—	1	0	0	0	0
	Non-infested	—	0	2	1	0	—	—	0	0	0	0	0	0	—	1	0	4	5	0	1	—	—	—	2	0	1	5	3
		—	0	2	0	0	—	—	0	0	0	0	0	0	—	0	0	0	0	0	0	—	—	—	0	3	5	1	1
		—	0	0	0	0	—	—	0	0	0	0	0	0	—	0	0	0	0	0	2	—	—	—	1	1	1	1	1
		—	0	0	0	0	—	—	0	1	0	0	0	0	—	0	1	0	4	0	0	—	—	—	0	1	4	4	3
		—	0	0	0	0	—	—	0	0	0	0	0	0	—	0	0	0	0	0	0	—	—	—	0	1	1	1	3
Flat B—Sterile	Infested	—	1	19	3	0	—	—	1	1	0	0	0	1	—	7	3	7	7	0	1	0	—	—	0	0	1	1	0
		—	5	27	3	1	—	—	1	0	1	0	1	0	—	3	3	1	1	0	0	—	—	—	0	0	0	0	0
		—	4	21	3	0	—	—	0	0	0	0	0	2	—	1	1	10	2	0	0	—	—	—	0	0	0	0	0
		—	1	13	4	1	—	—	2	0	0	0	0	3	—	10	6	5	2	0	1	—	—	—	0	0	0	0	0
		—	1	19	5	0	—	—	2	0	0	0	1	2	—	7	2	1	1	0	0	—	—	—	0	1	0	0	0
	Non-infested	—	0	1	0	0	—	—	0	0	0	0	0	1	—	11	5	12	4	2	0	—	—	—	1	0	0	0	0
		—	0	1	0	0	—	—	0	0	0	0	0	0	—	4	3	8	11	4	3	—	—	—	3	0	0	0	0
		—	0	0	0	0	—	—	0	0	0	0	0	0	—	0	0	0	2	2	1	—	—	—	12	4	4	3	3
		—	0	0	0	0	—	—	0	0	0	0	0	0	—	0	0	0	0	0	0	—	—	—	0	0	0	0	0
		—	0	0	0	0	—	—	0	0	0	0	0	0	—	0	0	0	0	0	0	—	—	—	0	0	0	0	0

which tended to be drier, being at the end of the flat. In both cases the amount of total killing was even higher in the recently invaded soil, whether sterile or non-sterile, than in the originally infested soil. The germinability of the seed in non-infested soil was found, from the first plantings in this experiment and the next, to be 94%. Thus any marked increase in non-emergence over three plants per row may be attributed to pre-emergence killing by the pathogen. Pre-emergence blight was evident in the second test in those rows that had been reached by the pathogen by the end of the first test.

Experiment II

The procedure of this experiment was identical with that in Experiment I except that the times of planting were varied in order to find whether the pathogen was able to invade non-infested soils in the absence of its host. For this reason the first planting was delayed until June 13, or nine days after the soils were allowed to come in contact with one another. Also, one month elapsed between the first and second plantings.

The results obtained in this case are shown in Table IX and Fig. 9. In the first trial the pathogen advanced only to the first row in the non-sterile soil in Flat C. A spot of infection from contamination also occurred in Row 9. Likewise only the first row in the sterile soil in Flat D was affected during this

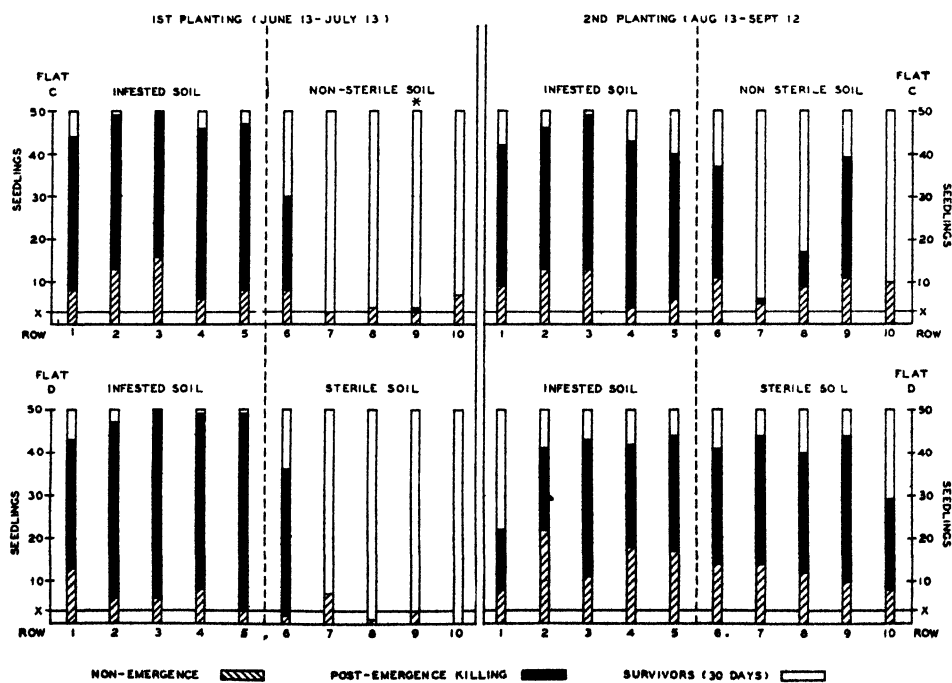


FIG. 9. Histogram showing the advance made by *P. parasitica* from infested soil into sterile and non-sterile soils as indicated by the number of seedlings killed in each row. First planting nine days later than in Fig. 8; second planting 30 days after the end of the first test period.

* Surface contamination.

test. Evidently the pathogen did not advance into the non-infested soils until the seedlings had been growing in it for some time, since in both experiments about three weeks elapsed between the first planting and the advance of the disease from the infested half of the flats. This period was not shortened by the 9-day delay in planting in Experiment II. It is difficult to explain why the pathogen did not advance beyond the first row in the non-infested soil in this trial.

Although one month elapsed before these flats were replanted, the disease at first affected only the area it had covered by the end of the first test. It spread progressively thereafter, reaching Row 9 in the non-sterile soil in Flat C, and affecting all rows in the sterile soil in Flat D by the end of the 30-day test period. The spot infection noted in Row 9 at the end of the first trial increased considerably during the second trial. The amounts of emergence in all rows in the non-infested soils were low after the second planting, but this was evidently caused by some factor other than the pathogen since post-emergence killing did not follow at once but advanced progressively from Row 6.

This experiment indicates an inability of the pathogen to migrate through the soil in the absence of its host. Its advance was not checked in the interim by lack of moisture, since the flats were well watered. Evidently its advance depends upon the presence of living roots of tomato plants. It may not necessarily have to parasitize these roots in order to advance. Abundant mycelium with sporangia has been observed along the surface of roots of seedlings growing in water without any evidence of penetration or breakdown in the host tissues (Plate II, Fig. 16). Also, the presence of roots of seedlings in liquid culture medium was found to stimulate the growth of the fungus. It is quite possible, therefore, that some by-product of the host that is essential for the development of the pathogen is present in the rhizosphere. This by-product may be a growth promoting substance such as auxin. If the pathogen does migrate along living roots, then the delay in its advance into non-infested soil may be accounted for by the time taken for the seedlings to develop root systems which intermingle.

INFLUENCE OF VARIOUS PLANT RESIDUES IN THE SOIL ON THE AGGRESSIVENESS OF THE PATHOGEN

One of the factors that influence the microbiological balance of a soil is the available organic substrate. Those micro-organisms that can best use the substrate in a given soil will predominate in that soil. If the substrate is changed, the balance will be shifted in favour of other organisms that are better able to use the new substrate. One way of changing the substrate is to add plant residues to the soil. The direction in which the microbiological balance becomes shifted will depend upon the type of decomposition that this plant material undergoes. Certain plants undergo protein degradation and release organic nitrogen, while other plants yield carbohydrates on decomposition. Thus one of two distinct groups of organisms will be favoured by

TABLE IX—*Concluded*

INVASION OF STERILE AND NON-STERILE SOILS BY *P. parasitica* AS SHOWN BY THE PROGRESSIVE DAMPING-OFF OF SEEDLINGS
DAILY LOSSES UP TO 30 DAYS FROM PLANTING—*Concluded*

Soil	Row	Number of days from planting																																
		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30										
		Second planting																																
Flat C—Non-sterile Infested Non-infested	1	1	0	2	—	3	0	0	1	2	1	1	—	7	1	0	1	0	—	2	3	4	3	1										
	2	3	0	3	—	6	1	2	2	1	0	—	7	1	0	1	1	0	—	1	2	1	0	1										
	3	1	1	6	—	13	1	0	0	0	2	—	—	4	3	2	1	0	—	2	0	0	0	0										
	4	2	0	4	—	6	4	2	3	2	1	—	—	4	0	2	2	1	—	1	3	0	0	1										
	5	1	0	4	—	8	4	4	1	1	3	—	—	4	1	1	0	0	—	0	1	1	0	0										
	6	3	0	5	—	1	1	1	0	1	0	—	—	4	2	1	2	0	—	3	1	0	0	1										
	7	0	0	0	—	0	0	0	1	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0										
	8	0	0	0	—	0	0	0	0	0	0	—	—	0	0	1	0	0	—	2	0	4	0	1										
	9	0	0	2	—	5	1	0	0	3	1	—	—	4	5	0	4	1	—	0	2	0	0	0										
	10	0	0	0	—	0	0	0	0	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0										
Flat D—Sterile Infested Non-infested	1	1	0	0	—	2	0	2	0	0	0	—	—	5	1	0	0	1	—	1	0	0	0	1										
	2	1	0	0	—	2	0	2	0	1	0	—	—	5	2	3	1	0	—	1	0	0	0	0										
	3	0	0	0	—	4	5	1	0	2	2	—	—	16	0	1	0	0	—	0	1	0	0	0										
	4	0	0	1	—	2	0	1	0	1	0	—	—	8	2	2	3	1	—	2	0	1	0	0										
	5	0	0	0	—	3	2	0	0	1	0	—	—	12	2	2	2	0	—	2	0	1	0	0										
	6	1	0	0	—	1	1	2	0	2	0	—	—	8	3	1	0	2	—	5	0	0	0	1										
	7	1	0	0	—	5	1	2	1	2	0	—	—	7	2	6	2	0	—	1	0	0	0	0										
	8	0	0	2	—	7	1	2	1	1	1	—	—	6	2	0	4	1	—	0	0	0	0	0										
	9	0	0	0	—	1	1	0	0	0	0	—	—	6	9	2	5	2	—	3	1	4	0	0										
	10	0	0	0	—	1	0	0	0	1	0	—	—	5	0	1	1	0	—	1	4	6	1	0										

a given plant residue. The group that dominates will tend to suppress the activity of the other group.

To study the effect of plant residues on the activity of *P. parasitica* in the soil the following experiment was carried out. Equal amounts of various plant residues were added to flats of infested non-sterile soil. This soil had been used in previous experiments and the pathogen had become well established. The residues were stems and leaves of fresh corn, soybean, timothy, and tomato plants. This material was run through a meat chopper and about 1500 cc. of each were mixed thoroughly with each flat of soil. The same day 500 tomato seeds were planted in each flat and in one check flat of infested soil. Records of the numbers killed each day were kept for 30 days, then the survivors were removed and counted. A second 1500 cc. portion of fresh residue was added to each flat which was then replanted immediately. On completion of this test, 30 days later, a third test was made without the addition of further residues. This time an inorganic nutrient solution, containing potassium acid phosphate, calcium nitrate, magnesium sulphate, and ammonium nitrate, was supplied to overcome the nitrogen deficiency that was apparent in some of the flats in the previous test.

The results of these tests are presented in Table X. There was little difference in the disease trends at first. In the first test the amount of total killing was slightly lower in the flats that received timothy and tomato residues than the others which were essentially the same as the check. In the second planting the timothy flat showed the greatest amount of disease, while the least killing occurred in the flat with soybean residue. In the third

TABLE X
EFFECT OF VARIOUS PLANT RESIDUES IN THE SOIL ON DISEASE INCIDENCE

Test No.	Residue added	Survivors, 30 days	Emergence		Damping-off			
			Total	%	Pre-emergence	Post-emergence	Total	%
1	Check	67	256	51.2	244	189	433	86.6
	Corn	82	314	62.8	186	232	418	83.6
	Soybean	72	285	57.0	215	213	428	85.6
	Timothy	116	334	66.8	166	218	384	76.8
	Tomato	114	313	62.6	187	199	386	77.2
2	Check	181	448	89.6	52	267	319	63.8
	Corn	200	349	79.8	151	149	300	60.0
	Soybean	269	400	80.0	100	131	231	46.2
	Timothy	115	360	72.0	140	245	385	77.0
	Tomato	193	377	75.4	123	184	307	61.4
3	Check	175	313	62.6	187	138	325	65.0
	Corn	155	244	48.8	256	79	335	67.0
	Soybean	294	369	73.8	131	75	206	41.2
	Timothy	183	290	58.0	210	107	317	63.4
	Tomato	191	301	60.2	199	110	309	61.8

trial the soybean flat showed a definite reduction in both pre- and post-emergence killing. The other flats showed essentially the same degree of disease incidence as the check flat containing no residue. Apparently either the products of the breakdown of the soybean plant or the microbiological activity associated with it retard the aggressiveness of the pathogen. Of the residues used, soybean is the only one that yields carbohydrate and not organic nitrogen on decomposition. It may be that certain carbohydrates favour a group of organisms that suppress the activity of *P. parasitica* in the soil. With abundant organic nitrogen as a substrate, these suppressing organisms may not be dominant but may give way to another group with which the pathogen is compatible. The residue of the normal host of the pathogen does not appreciably favour or suppress its aggressiveness.

Substantiation for this hypothesis was sought in the reaction of the pathogen to the addition of organic nitrogen, in the form of gelatine, and a carbohydrate, dextrose, to infested soils, but the results of these limited tests were inconclusive. Further experiments along this line are desirable.

Host Range and Varietal Resistance

Natural infection has not been observed on any host other than varieties of *Lycopersicon esculentum* Mill. However, artificial inoculations have revealed a wide range of potential hosts, almost exclusively within the family Solanaceae.

In testing a plant as to the susceptibility of its stem to this disease, usually a cutting was taken and placed with the stem immersed in three to four inches of water. The inoculum, in the form of mycelium bearing sporangia, or of a zoospore suspension, was then added to the water. After a few days at room temperature the stem of a susceptible host showed a brownish lesion extending from the water line upwards an inch or more. If the tissues of the stem collapsed in the discoloured region (Plate I, Fig. 13), the plant was considered to be susceptible. This method of testing plants for resistance or susceptibility does not closely simulate the conditions under which natural infections occur, and the environmental factors all favour the attack by the parasite on the host. However, plants that resist the disease under these conditions may be safely assumed to resist it under conditions less favourable for attack.

In some cases the reaction of leaves or fruits was tested by placing drops of zoospore suspension on the organ to be tested and keeping it in a moist chamber. Drops of sterile water were placed on similar organs that served as controls.

More than 40 commercial varieties of *Lycopersicon esculentum* Mill. were tested in this way. All showed complete susceptibility of stems with no appreciable difference between varieties.

The following species of *Lycopersicon* were also tested:

- (1) *Lycopersicon esculentum* var. *cerasiforme* (Dun.) A. Gray (*Lycopersicum humboldtii* Dun.)
- (2) *Lycopersicon hirsutum* Humb. & Bonpl.
- (3) *Lycopersicon peruvianum* (L.) Mill.
- (4) *Lycopersicon peruvianum* var. *dentatum* Dun. (*Lycopersicum chilense* Dun.)
- (5) *Lycopersicon pimpinellifolium* (Jusl.) Mill.
- (6) *Lycopersicum racemiflorum* Dun.*
- (7) *Lycopersicum racemiforme* Lange*
- (8) *Lycopersicum racemigerum* Lange*
- (9) *Lycopersicon rhombifolium* Dipp.

The leaves of all species proved susceptible as did the stems of all except *L. hirsutum*, *L. peruvianum* var. *dentatum*, and *L. rhombifolium*. These three displayed a perceptible degree of resistance, though they were not immune. More extensive tests were made with *L. rhombifolium*. These showed that the same degree of resistance was inherited by the F_1 and part of the F_2 generations of a cross between it and *L. esculentum* var. *Vetomold*. The degree of resistance, however, was not sufficient to warrant continuing this phase of the problem for the purpose of breeding a tomato variety resistant to *Phytophthora parasitica*.

The tests were next extended to include as many plants belonging to the family Solanaceae as were available. Mature and young leaves and stems were used in most cases. In 16 of the 28 species tested, the leaves only were susceptible; in five only the young leaves were susceptible; while in the remaining seven all the leaves were resistant. In only five of the 19 species tested were the stems susceptible, the other 14 showing no reaction. In no case was a stem found to be susceptible when its leaves were resistant, but in many cases the leaves were attacked when the stem was not. The Irish potato is a striking example of the latter condition. Its leaves and petiole were as susceptible as tomato leaves, but all attempts to induce infection in stems by inoculating with zoospores or with mycelium in wounds failed.

The fruits of *Nicandra Physalodes* L., *Solanum Melonga* L., *S. nigrum* L., and *S. Pseudocapsicum* L. were tested and found to be susceptible to attack by germinating zoospores. Fruits of *Capsicum annum* L. resisted this form of attack but were rotted by mycelium of the fungus placed in wounds.

While potato stems are immune to the disease, a tuber can be readily infected by placing drops of zoospore suspension on the surface or by inserting mycelium into wounds. The fungus develops quite rapidly, invading about two-thirds of the tuber within one week and causing the pink rot characteristic of certain *Phytophthora* species. The colour is not evident when infected tubers are freshly cut, but after exposure to the air for about 20 minutes a salmon pink colour develops over the area invaded. This area later becomes dark brown.

* According to Muller (18) these species are synonymous with *Lycopersicon pimpinellifolium*.

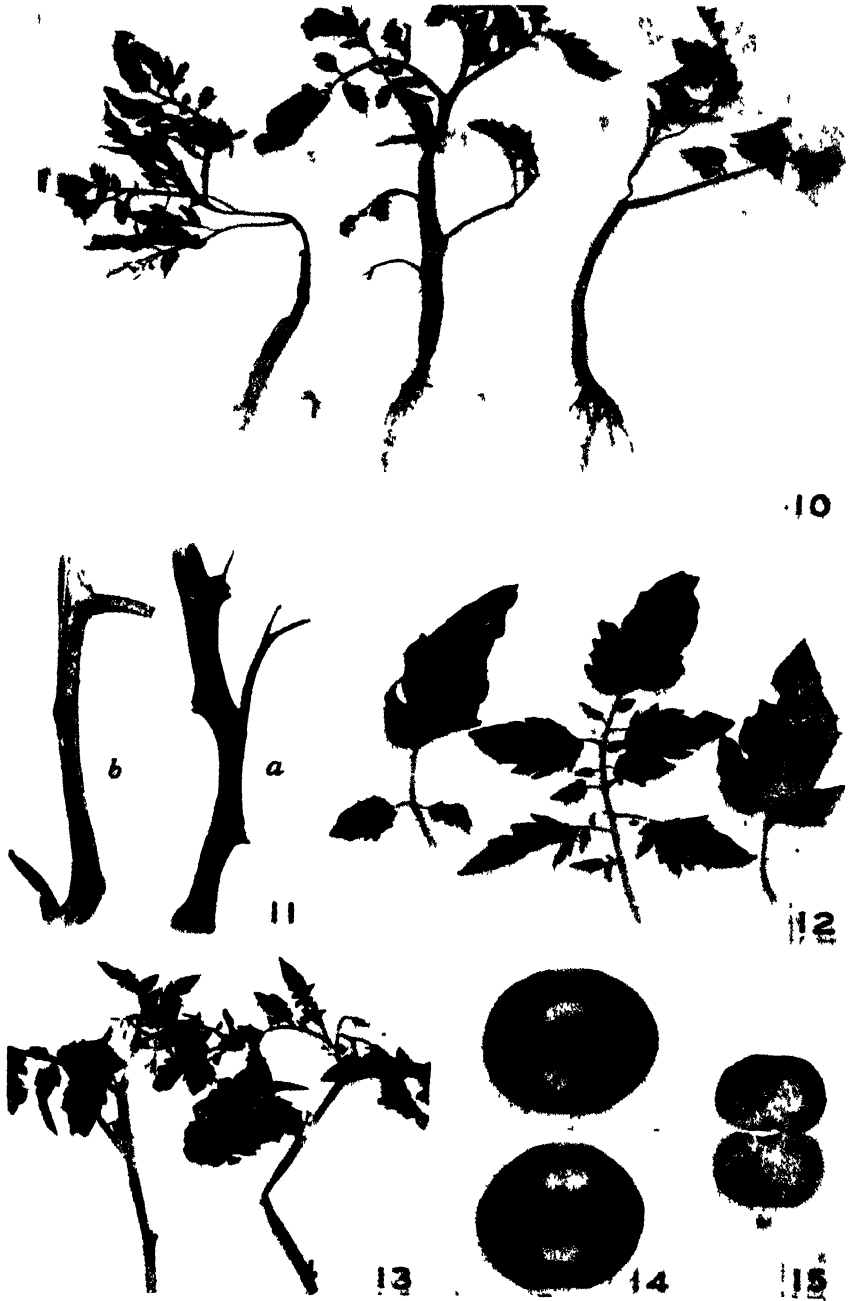


FIG. 10. Typical lesions on stems of 6-weeks-old tomato plants naturally infected with *Phytophthora parasitica*.

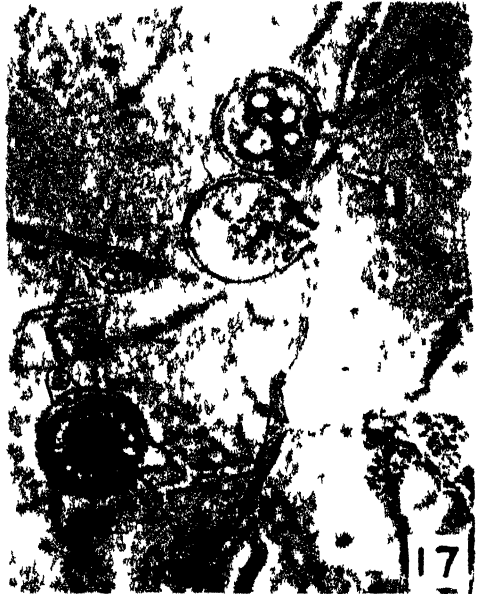
FIG. 11. Extensive lesions on stems of mature plants: a, surface view; b, section showing breakdown of parenchymatous tissues.

FIGS. 12 TO 14. Tomato leaves, cuttings, and fruits, three days after inoculating with zoospores.

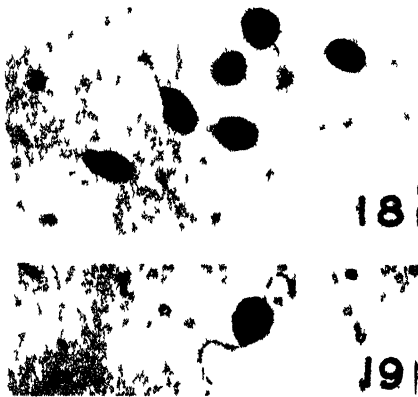
FIG. 15. Naturally infected fruit cut open to show depth of lesion.



16



17



18



19



20



FIG 16 Sporangia of *Phytophthora parasitica* formed in association with a rootlet of a tomato seedling growing in water

FIG 17 Sporangia from culture on oatmeal agar ca 430×

FIGS 18, 19 Zoospores killed with osmic acid and stained with cotton blue ca 865×

FIG 20 Oospore formed on rootlet of tomato seedling growing in water ca 260×

FIG 21 Chlamydospores from culture on oatmeal agar ca 260×

A limited number of tests outside of the family Solanaceae revealed no susceptible host, with the single exception that the fungus caused decay in apple fruits following wound inoculations. Plants that showed no reaction included snapdragon, cucumber, dahlia, buckwheat, and garden bean.

The seedling reaction of a few plants to the parasite was tested by planting seeds in infested soil. Although the stem and leaves of petunia had proved to be immune, the seedlings of this plant damped-off readily. Seedlings of carrot, lettuce, radish, and pea were not affected. It was observed that young seedlings of chickweed (*Stellaria media* (L.) Cyrill) growing in a plot of infested soil in the field damped-off, apparently due to *P. parasitica*.

Several features of the infection capabilities of this isolate of *Phytophthora parasitica* are shown by these tests. It has a possible host range extending fairly generally throughout the family Solanaceae. It can attack storage organs such as fruits and tubers when other parts of the plant are immune. It parasitizes leaf tissue more readily than stem tissue. It can often infect young tissues when older tissues of the same type are resistant. This age resistance on the part of the host is shown in many cases by the difference in reaction between young and old leaves, and by the fact that only young succulent stems of tobacco can be infected.

Control

Since this disease is caused by a soil-borne parasite, the following measures would be expected to contribute to its control: (1) resistant varieties of host; (2) sanitation; (3) rotation of crops; (4) chemical seed treatments; and (5) soil disinfestation.

(1) *Resistant Varieties*

The most desirable form of control for this disease would be the development of a good commercial variety of tomato that would thrive in the presence of the pathogen, even under conditions favouring infection. However, the prospect of developing such a variety is not encouraging. While some resistance was found in certain wild *Lycopersicon spp.*, it was not a high degree of resistance even in mature plants and was apparently inoperative in their seedling stage.

(2) *Sanitation*

Unfortunately the presence of the pathogen is not detected until its effect on growing plants becomes apparent. While it is impossible at that time to rid the soil of the pathogen without injury to the crop, certain methods may be employed to reduce its destructive attacks. Remembering that it is soil-borne and that its spores are dependent upon water for their spread, one should avoid excessive watering and take every precaution to avoid splashing the soil onto the stems. Mulching the surface of the soil with straw will reduce the danger of this. So far as possible one should control the atmosphere of greenhouses so that it does not favour infection. In other words, greenhouses should be kept as cool and as dry as is feasible for satisfactory growth

of the plants. Dead plants should be carefully removed and destroyed. The tracking of soil from one house to another should be avoided as far as possible.

(3) *Rotation of Crops*

Whenever it is feasible, it is wise to alternate tomatoes with other greenhouse crops. Continuous culture of the same plant tends to increase the degree of infestation of the soil by parasitic organisms specific to that host. *P. parasitica* is not likely to be soon "starved out" by this practice, since it has been found to be still active in soil in which no susceptible host has been growing for at least a year. The degree of infestation may be temporarily reduced, however, to the point where the disease does not seriously interfere with production.

(4) *Seed Treatments*

Chemical seed treatments have been reported by various investigators to control damping-off of seedlings. Horsfall (11) recommended red copper oxide, zinc oxide, and copper sulphate for protecting seedlings from *Pythium*, *Rhizoctonia*, and *Phytophthora*. Semesan, a mercury compound, has also been recommended. Seed lots treated with these chemicals, as well as with copper chloride, copper carbonate, copper tartrate, copper naphthalate, copper acetate, and Copoloid (a colloidal copper compound) were planted in soil infested with *P. parasitica*. Some of the treatments, especially the Semesan, interfered seriously with the germination of the seeds. Most of the treatments reduced the amount of pre-emergence blight of the seedlings, but none of them gave adequate protection against postemergence killing. Since this is the more destructive phase of this disease, control by means of chemical seed treatment does not appear to be feasible. Moreover, since the parasite can attack plants at any stage, even to maturity, it is useless to try to protect the seedlings in the seed bed, then carry the fungus in the soil around their roots into the cold frame or greenhouse. How a little inoculum of this sort is sufficient to cause serious trouble was demonstrated by the experiments on the establishment and spread of the pathogen in the soil.

(5) *Soil Disinfestation*

The only completely effective means found for protecting tomato plants of all ages growing in seed beds, cold frames, or greenhouses is to disinfest the soil by some means of sterilization. The accepted practices of steam and formaldehyde treatments were found to be 100% effective. If it is available, steam is preferable, since planting can be done within a short time without danger of a chemical residue injuring the plants.

Two newer sterilizing agents not generally used were tried and found to be very effective. The first of these was calcium cyanamide, known commercially as Aero Cyanamid. To one flat of infested soil 20 gm. of pulverized Cyanamid were added and thoroughly mixed; to a second flat 40 gm. were added; a third flat was kept as a check. The above quantities are roughly equivalent to 1000 and 2000 lb. per acre respectively. No appreciable change

in the acidity of the soils was detected, all three lots showing a pH of 7.2 when tested three weeks after mixing. The flats were heavily watered for two weeks after the addition of the Cyanamid before they were planted with 500 seeds each.

Twenty-five days after planting the results were as follows:

Check flat:	340 germinated, 204 alive.
20 gm. Cyanamid:	409 germinated, 409 alive.
40 gm. Cyanamid:	391 germinated, 391 alive.

While both concentrations of Cyanamid controlled the disease, the higher concentration delayed germination greatly and caused considerable burning of the foliage, even to the extent of being lethal in many cases. The lower concentration delayed germination somewhat, but injury was confined to very slight burning at the tips of the leaves. The seedlings were a healthier colour and more vigorous than those in the check flat, apparently as a result of the increased nitrogen available. Perfect control without any injury could probably be obtained by waiting longer before planting or by using less Cyanamid.

The second chemical used to disinfest the soil was trichloronitromethane (CCl_3NO_2). This is known commercially as Larvacide, Chloropicrin, or "tear gas". One cubic foot of heavily infested soil was placed in a tightly closed wooden box. Two holes were made in the soil and $1\frac{1}{2}$ cc. of liquid chloropicrin were poured into each. The holes were then filled and the surface of the soil was wetted to seal the gas in. The top of the box was sealed by pasting tarred paper over the cracks. After three days the lid was removed and the soil stirred to allow the gas to escape. Two weeks later one flat of this soil was planted with 500 tomato seeds, as was a check flat of infested soil.

Three weeks later the treated flat had 431 living seedlings and no dead ones. Only 120 of those in the check flat survived. Pre-emergence blight was also reduced, although the exact amount was not determined.

Both of these methods have possibilities as commercial treatments. Both are claimed by their manufacturers to kill nematodes and weed seeds as well. Cyanamid is in addition a fertilizer, but heavy doses unbalance the normal chemical composition of the soil and it is difficult to remove the excess which is injurious to plants.

Discussion

Several species of *Phytophthora* have been reported to be the cause of various diseases of tomatoes. *Phytophthora cryptogea* Pethyb. & Laff. was described by Pethybridge and Lafferty (20) in 1919 as the cause of a foot rot, or collar rot. *P. infestans* (Mont.) de Bary, which causes late blight of potato, was shown by Reed (22) in 1912 to cause a similar blight in tomato. Mills (17) in 1940 demonstrated the relationship between the potato and tomato strains of this species. *P. mexicana* Hotson & Hartge was isolated from tomato fruits by Hotson and Hartge (12) in 1923. *P. terrestris* Sherb.

was described by Sherbakoff (25) in 1917 as the cause of "buckeye rot" of tomato fruits. Butler (4), Bewley (2), Ashby (1), Leonian and Geer (15), and Tucker (26) agree that this species is identical with *P. parasitica*. Zonate markings have not been observed in fruits infected with the organism used in the present study, and tomato stems inoculated with a *Phytophthora* species isolated from fruits showing typical buckeye symptoms did not become infected. Reddick (21) described a "stem-girdle" disease of tomatoes which affected seedlings, stems, foliage, fruit, and roots. The causal organism was identified by Tucker (26) as *P. parasitica* Dast. Reddick's description coincides very closely with that of the disease under investigation here. There are, however, differences in symptomatology, life history, and host range that suggest that the causal organisms are different strains of the same species. In the first place, root infections were much less severe and never resulted in the death of the plant in the present study. Secondly, Reddick reported that oospores developed sparingly on oatmeal agar. In three years of culture on various media, including oatmeal agar, no sexual stage has been found for any of the isolations made from tissues affected by the disease herein described, although on one occasion a few oospores were found in association with sterile roots of seedlings placed in infested water. Finally, Reddick reported successful inoculations of stems of bean, cucumber, and young egg-plants, whereas in the present study such inoculations failed to produce lesions.

The restricted occurrence of the disease raises an interesting question: Is the pathogen likewise limited in its distribution, or is it ubiquitous, but destructive only under a favourable combination of conditions? Referring to *P. parasitica* causing buckeye rot, stem girdle, and damping-off of tomatoes, Tucker (27) states that "distribution is probably general in the Gulf and South Atlantic states." A general distribution cannot be postulated for Ontario, although the pathogen can apparently survive in some soils here. Under normal conditions even where the organism is present in the soil this must be brought into the greenhouse before the fungus becomes aggressive. The studies reported here on temperature and moisture in relation to disease incidence account in part for this situation. In many of the local outbreaks an effort was made to trace the source of infestation. In every case it could be traced, directly or indirectly, to soil brought in from the field and used in the compost. In one case it was suspected that the organism was introduced with a load of muck soil, but it is not always a dominant inhabitant of such soil, since seedlings planted in muck from two different districts were not affected. In other cases the trouble began when grass sod was used in the compost. Sods of four different grass types were tested by growing tomato seedlings on them, but again no infection occurred. If the pathogen was present in these soils it must have been held at a very low level of aggressiveness by competitive and environmental factors under natural conditions. Greenhouse conditions are so unnatural, however, not only with respect to temperature and moisture but also to substrate, that the microbiological equilibrium must be changed drastically, and the new conditions may be

such as would favour the pathogen. Moreover, the common practice of raising two crops of tomatoes a year without rotation favours micro-organisms parasitic on this host.

The strain of *Phytophthora parasitica* causing this tomato disease is better able to compete with other micro-organisms in the soil than many of the soil-borne parasites that have been investigated in this respect. Henry (8) found that even a trace of non-sterile soil added to soil infested with *Helminthosporium sativum* P.K. and B. brought about a marked reduction in the severity of the foot rot disease in cereals, and that if larger amounts of non-sterile soil were added he was unable to recover the pathogen. The reverse situation is true of *P. parasitica*: a trace of infested soil is sufficient to cause a large amount of non-sterile soil to become infested. This parasite is not only more successful in establishing itself in the soil, but also in maintaining a level of activity that renders it destructive to its host over longer periods of time than many other parasites. For example, Sanford and Broadfoot (24) found that, in greenhouse culture, inoculum of *Ophiobolus graminis* that destroyed the first planting of wheat with take-all disease was wholly ineffective after 120 days on the second planting. The decline in degree of infestation of a soil by *P. parasitica* could be detected by repeated plantings of tomato seedlings, but the pathogen was still aggressive after one year in the soil. Furthermore, workers have frequently failed to get infection in the field when inoculum was applied to the soil, whereas *P. parasitica* has been shown to be virulent in field tests as well as in greenhouse experiments.

The effect of competition on the activity of *P. parasitica* is, nevertheless, appreciable, as shown by various experiments reported here. More information concerning the relationships of the other soil micro-organisms to this pathogen is highly desirable, though rather difficult to obtain. Henry (8) found that adding mixtures of bacterial cultures isolated from Edmonton black loam to soil infested with *Helminthosporium sativum* reduced foot rot disease in wheat slightly; soil actinomycetes suppressed the disease more than the bacteria; saprophytic soil fungi reduced the disease most of all and the pathogen could not be recovered from the soil 24 days after these were added to the soil. He attributed the reduction in the activity of *H. sativum* to exhaustion of food by the other soil fungi. Sanford and Broadfoot (24) studied the effects of fungi and bacteria isolated from the soil individually as to their ability to suppress *Ophiobolus graminis*. Of 26 species of fungi isolated, six gave complete protection of wheat seedlings, seven gave partial protection, while the remainder had no effect. Of 40 species of bacteria tested, 15 suppressed the parasite completely, eight gave partial protection, and the remainder had no effect. They also tested the filtrates from these cultures and found that some of them suppressed the virulence of the pathogen, though less effectively than living cultures. They believed that the toxicity of by-products of fungous and bacterial growth was the chief factor concerned. Broadfoot (3) cultured the same organisms with *O. graminis* Sacc. on potato dextrose agar and other media and found that some were antagonistic

to its growth while others were compatible. The former were not always the same ones that suppressed the activity of the pathogen in the soil, however. He thought that this difference in reaction might be accounted for by the fact that various micro-organisms, each with its complement of enzymes, produce substances that differ in kind and amount according to the substrate.

The effect of various plants residues on the aggressiveness of *P. parasitica* is in agreement with the observation of various workers that the microbiological equilibrium of a soil may be modified by changing the organic substrate. It is known that the addition of green manure to the soil brings about a striking increase in the bacterial population. The addition of green rye to certain soils has been found to reduce the amount of potato scab caused by *Actinomyces scabies* (Thax.) Güssow. Millard and Taylor (16) demonstrated that scab incidence was markedly reduced by the addition of grass clippings if the obligately saprophytic species *A. praecox* Millard & Burr was also present in the soil. The two species are incompatible in culture, the parasite making slower growth and apparently being starved by the saprophyte. Waksman and Hutchings (28) demonstrated associative and antagonistic relationships between micro-organisms concerned with the decomposition of plant residues. They showed that the type of decomposition is a function of the organisms of a mixed population that are in a position to attack the introduced substrate.

It would be of interest to know the effects of various oxygen and carbon dioxide tensions on the growth of *P. parasitica* as well as on the microfloral population as a whole. Garrett (6) postulated that high carbon dioxide concentrations, built up by the respiration of the micro-organisms and the roots of higher plants in the soil, constitute a primary factor in the suppression of *Ophiobolus graminis* by organisms better able to develop under those conditions.

The apparent dependence of *P. parasitica* on living roots of its host for migration through the soil is highly suggestive. Padwick (19) showed that *Ophiobolus graminis* spreads little, if at all, in bare soil, but it spread 12 inches in six weeks in soil in which susceptible grasses were growing. It apparently spread actively through the soil only by root contact, passing from plant to plant by means of the fine network of roots. Garrett (6) demonstrated, furthermore, that the spread of the fungus is along the outside of wheat roots, internal development being sharply restricted. *P. parasitica* was found in these studies to grow profusely around the roots of seedlings growing in water, and to be stimulated in its growth in liquid culture by the presence of rootlets. The soil in the immediate vicinity of plant roots or the rhizosphere is known to support a much higher microfloral population than that beyond the zone of the plant's influence. West and Lochhead (30) have shown that there is a qualitative as well as a quantitative difference here. They found that those bacteria that require the growth substances thiamin and biotin as well as amino nitrogen are favoured in the immediate vicinity of the roots. West (29) also demonstrated that the young roots of higher plants excrete significant amounts of thiamin and biotin normally, even under sterile conditions. Many

fungi, especially Phycomycetes, have been shown by Robbins (23) to respond to certain growth promoting vitamins, including thiamin. Leonian (14) found that substances, which he believed to be auxins, excreted by corn root tips, stimulated the growth of *Phytophthora cactorum* (L. and C.) Schroet. in culture. The production of some such substance or substances by the roots of tomato seedlings may well be the cause of the association observed between *Phytophthora parasitica* and the roots of its host.

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The author wishes to express his sincere gratitude to Professor D. L. Bailey for suggesting and directing this investigation; to Professor J. W. MacArthur for providing many of the *Lycopersicon* and *Solanum* species used; and to Professor E. F. Palmer for placing at his disposal the facilities of the Horticultural Experiment Station, Vineland, Ontario.

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AGRICULTURAL METEOROLOGY: SUMMER SEQUENCE OF MONTHLY MEAN TEMPERATURE AT WINNIPEG, SWIFT CURRENT, AND EDMONTON¹

By J. W. HOPKINS²

Abstract

An analysis of the monthly sequence of mean temperature during the summer period, April–September, of the years 1894–1937 has been made by expressing each annual sequence as an orthogonal polynomial function of time. Whereas the precipitation sequence, previously studied, required terms of the fourth or fifth degree for its adequate representation, the average temperature sequence at all three stations was very closely approximated by a third degree polynomial. On the average, corresponding coefficients for each station differ significantly, indicating decreasing continentality of the temperature regime with distance westward from Winnipeg. Annual variations in corresponding coefficients for the three locations are appreciably correlated, but for the most part exhibit no regular sequence in time. However, in recent years at Edmonton the mean temperature for April has tended to be lower, and that for July and August to be slightly higher, than previously. There is some suggestion of a feeble inverse correlation in the annual fluctuations of temperature and precipitation.

Introduction

In a preceding paper (3), the writer has described a statistical analysis of the monthly sequence of summer precipitation at three representative meteorological stations in the Prairie Provinces of Canada. It is now desired to present the results of a parallel analysis of monthly mean air temperature at the same locations, viz., Winnipeg, Manitoba (lat. 49° 53' N., long. 97° 7' W., alt. 760 ft.), Swift Current, Saskatchewan (50° 20' N., 107° 45' W., 2440 ft.), and Edmonton, Alberta (53° 33' N., 113° 30' W., 2158 ft.), and to compare certain characteristics of the temperature and precipitation sequences.

Data

As before, the primary data, namely the mean air temperatures in degrees F. recorded at each station for the months mentioned were extracted from the published observations of the Meteorological Service of Canada (1). However, whereas the analysis of precipitation sequences comprehended the period 1890–1937, temperature records were incomplete in the earlier years;

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TABLE I
SEASONAL TEMPERATURE COEFFICIENTS, WINNIPEG

Year	a'	b'	c'	d'	e'	f'
1894	57.5	93	-219	-17	15	-43
1895	56.2	51	-112	-44	0	20
1896	55.7	92	-211	17	-3	-11
1897	57.2	141	-118	6	10	66
1898	56.0	126	-150	-4	-2	28
1899	55.5	121	-180	-24	6	6
1900	59.5	59	-138	-36	-8	-54
1901	57.8	75	-164	-40	-16	64
1902	55.7	118	-169	-27	-17	81
1903	55.3	74	-173	-11	-1	5
1904	54.3	108	-176	28	-10	2
1905	56.0	156	-126	-24	0	6
1906	58.8	121	-98	-54	28	-36
1907	51.7	186	-223	-59	31	-37
1908	56.8	125	-140	0	14	18
1909	56.5	189	-207	19	-5	-7
1910	57.5	95	-174	-40	38	-44
1911	56.7	78	-169	3	-3	-39
1912	56.3	78	-164	18	12	-18
1913	57.7	94	-121	-51	15	-75
1914	57.7	138	-193	13	9	71
1915	57.2	75	-79	-85	-13	1
1916	56.0	144	-180	-76	6	82
1917	55.7	150	-175	-15	7	51
1918	54.8	81	-164	-94	-2	-44
1919	59.8	95	-182	10	0	-16
1920	57.2	201	-190	26	-24	-20
1921	58.5	127	-195	17	17	-11
1922	59.7	102	-151	27	-19	-9
1923	58.0	152	-207	57	23	15
1924	53.7	152	-154	-68	14	8
1925	58.0	112	-126	-88	0	-14
1926	56.3	92	-185	-13	-23	73
1927	56.0	132	-135	-33	15	-3
1928	55.7	132	-184	12	-20	60
1929	56.3	126	-176	-104	4	-28
1930	58.7	124	-169	-101	11	-13
1931	58.8	143	-143	-37	19	-47
1932	58.5	125	-192	10	-2	-52
1933	58.8	137	-200	12	2	-30
1934	55.5	83	-186	-2	-12	32
1935	55.5	133	-195	-67	3	91
1936	58.0	174	-237	19	-11	137
1937	55.3	60	-119	5	7	19
Mean	56.8	117.5	-167.0	-20.8	2.6	6.5
Standard deviation	1.66	36.69	34.72	40.08	14.34	46.64
"t"	-	21.836**	31.905**	3.442**	1.209	0.921

** Exceeds 1% level of significance.

and the study of these accordingly had to be confined to the period 1894-1937. Again as before each observed six-monthly sequence was expressed by the use of Fisher and Yates' multipliers (2) as a fifth degree orthogonal polynomial function of time, the coefficients of which are listed in Tables I, II, and III.

TABLE II
SEASONAL TEMPERATURE COEFFICIENTS, SWIFT CURRENT

Year	a'	b'	c'	d'	e'	f'
1894	58.2	93	-193	-77	-5	-1
1895	55.5	43	-117	-97	-1	31
1896	55.5	97	-195	-53	17	11
1897	59.0	104	-129	-1	-23	25
1898	55.7	138	-172	-32	-12	8
1899	53.7	136	-148	-14	18	44
1900	59.0	26	-153	-29	3	-13
1901	56.3	48	-155	-87	-45	123
1902	54.7	92	-130	-28	-26	52
1903	54.2	69	-154	-46	14	-38
1904	55.0	110	-159	-15	-1	15
1905	55.3	140	-134	-70	-10	-14
1906	58.0	98	-102	-92	16	14
1907	51.2	163	-175	-57	7	-15
1908	56.8	93	-122	-32	12	62
1909	55.0	182	-171	17	-9	7
1910	56.3	40	-116	-50	30	8
1911	53.8	83	-152	8	6	-38
1912	55.0	52	-153	-53	3	-61
1913	57.2	81	-112	-54	14	-72
1914	57.5	103	-168	-62	14	92
1915	56.2	39	-73	-131	-23	-37
1916	54.3	110	-131	-75	13	57
1917	56.2	131	-178	6	-16	120
1918	57.0	102	-162	-58	4	-44
1919	60.0	94	-159	-41	-1	-19
1920	56.2	183	-193	-7	-19	31
1921	57.0	104	-210	-26	0	-52
1922	57.5	133	-126	-12	0	-42
1923	57.0	106	-168	-4	14	10
1924	54.5	131	-138	-34	6	22
1925	57.2	77	-136	-58	-2	28
1926	55.7	34	-181	-81	-11	81
1927	55.0	122	-144	-58	16	-26
1928	56.5	107	-159	37	-29	83
1929	55.5	137	-192	-98	-2	-16
1930	58.0	96	-129	-119	5	-13
1931	59.0	92	-156	-18	8	-18
1932	58.0	82	-132	-28	-4	-20
1933	57.7	116	-190	-24	4	-30
1934	57.3	28	-173	-47	-29	77
1935	54.5	137	-168	-38	14	68
1936	59.5	119	-210	-16	-14	112
1937	59.2	101	-157	-49	5	37
Mean	56.4	99.4	-154.0	-43.2	-0.9	14.8
Standard deviation	1.82	38.11	28.59	35.76	15.50	49.64
"t"	-	17.296**	35.726**	8.023**	0.379	1.970

** Exceeds 1% level of significance.

Analysis

Characteristics of Average Sequence

In contrast to the situation encountered in considering the rainfall sequence (3), the frequency distributions generated by the 44 annual values of the coefficients a' f' show only two significant departures from normality,

TABLE III
SEASONAL TEMPERATURE COEFFICIENTS, EDMONTON

Year	a'	b'	c'	d'	e'	f'
1894	53.7	58	-178	-62	-16	-20
1895	53.2	25	-130	-50	2	26
1896	52.7	102	-169	-13	11	19
1897	55.8	61	-110	6	-22	-6
1898	55.5	93	-141	-7	-17	13
1899	51.8	111	-125	1	17	11
1900	54.0	10	-114	10	-6	2
1901	52.5	51	-147	-49	-35	55
1902	52.8	65	-110	-50	-22	62
1903	51.7	68	-157	-7	5	-41
1904	53.7	64	-109	-41	9	17
1905	53.8	71	-104	-24	-4	24
1906	55.8	63	-98	-92	28	14
1907	50.2	125	-157	-15	19	-3
1908	54.7	70	-124	-10	-8	70
1909	52.8	147	-164	52	-2	14
1910	53.8	47	-113	-33	11	15
1911	52.7	74	-139	9	3	-9
1912	54.5	39	-129	-1	-9	-65
1913	54.3	74	-95	-41	9	-37
1914	54.3	76	-140	-54	0	18
1915	54.5	39	-84	-116	-28	-16
1916	52.7	76	-106	-34	4	4
1917	53.3	106	-161	41	-21	73
1918	53.3	92	-113	-53	11	-25
1919	55.2	77	-103	-53	-1	-7
1920	51.0	150	-198	0	-6	72
1921	53.0	54	-147	-11	7	-13
1922	54.8	103	-128	-12	-6	-6
1923	54.0	94	-132	-6	10	-12
1924	53.0	88	-144	-2	2	68
1925	53.3	44	-146	-26	-4	20
1926	52.3	20	-164	-80	-2	74
1927	51.8	113	-158	-52	2	-32
1928	52.7	94	-160	54	-18	72
1929	52.5	103	-174	-12	-4	-6
1930	54.5	83	-135	-87	1	-3
1931	54.2	59	-118	-46	-4	16
1932	55.7	88	-121	-7	-13	-23
1933	53.5	85	-147	-35	-21	-25
1934	53.0	8	-132	-42	-18	24
1935	52.3	136	-185	21	5	51
1936	54.5	93	-189	23	-21	55
1937	58.0	130	-192	-50	-16	26
Mean	53.6	77.9	-138.4	-24.0	-3.8	13.8
Standard deviation	1.43	33.63	28.28	36.44	13.47	34.51
"t"	-	15.371**	32.460**	4.370**	1.880	2.647**

** Exceeds 1% level of significance.

namely negative skewness in the case of a' at Swift Current and e' at Edmonton. It may accordingly be inferred that at the former station summer seasons of moderately above and appreciably below average mean temperature have been more frequent than those moderately below or appreciably above average,

respectively. At Edmonton there has been a similar uneven occurrence, not of the mean temperature for the six months as a whole but of the element of seasonal trend corresponding to the fourth degree polynomial term ξ_4' (2). As is noted below, however, this is not a salient feature of the average season. Apart from these two instances, the annual fluctuations would seem to range themselves symmetrically about the mean in each case.

Whereas the sequence of precipitation previously examined (3) required significant polynomial terms of the fourth or fifth degree for its adequate representation, the average temperature sequence was closely approximated by a third degree function, as is illustrated in Fig. 1. At all three stations this

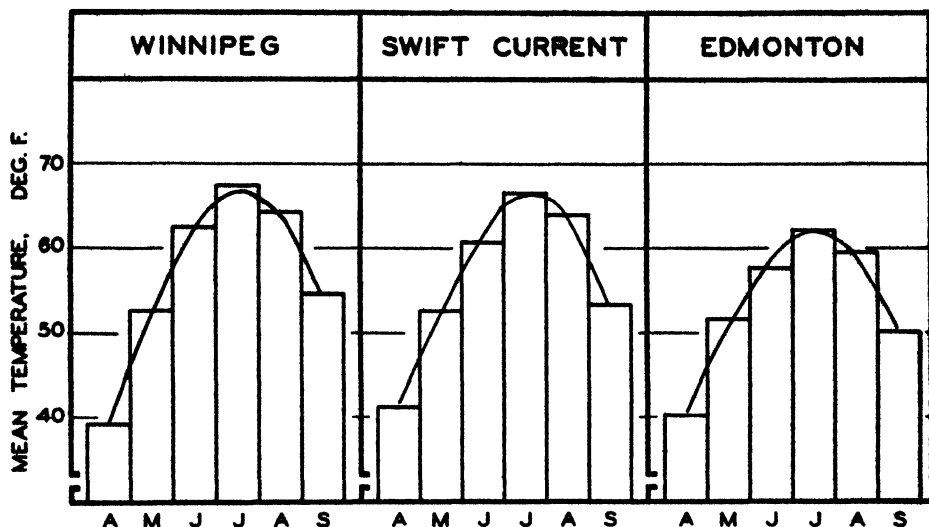


FIG. 1. Average summer sequence of monthly mean temperature at Winnipeg, Swift Current, and Edmonton, 1894-1937 (rectangular columns), and approximation to sequence by third degree polynomial (continuous curves).

average polynomial is characterized by a positive linear coefficient b' (arising from the fact that July, August, and September have a higher mean temperature than April, May, and June), a negative quadratic coefficient c' (monthly mean temperature at a maximum in July) and a small negative cubic coefficient d' (reflecting some asymmetry in the rate of increase and decrease in mean temperature before and after the July maximum). However, although the average seasonal trend is thus of the same general nature at each of the three locations, the analyses of variance summarized in Table IV demonstrate that the average numerical magnitude of each of the trend coefficients mentioned above (as well as that of the general mean a') differs between stations by a statistically significant amount. On the average, both b' and c' are largest at Winnipeg, smallest at Edmonton, and intermediate at Swift Current (Tables I to III). This may be associated with decreasing "continentality" of the temperature regime with distance westward from Winnipeg, the station most centrally situated with respect to the land mass.

TABLE IV
ANALYSIS OF VARIANCE OF TEMPERATURE COEFFICIENTS

Variance	Degrees of freedom	Mean square			
		a'	b'	c'	d'
Between stations	2	135 0500**	17,261.8**	9029.4**	6490.5**
Between years	43	6 1474**	3237.0**	2220.5**	3089.6**
Remainder	86	0.9743	310.1	320.7	561.2

** Exceeds remainder, 1% level of significance.

The average of the mean temperatures for the six months (a') at Edmonton is significantly lower than that recorded at either of the other two points.

Annual Variations

In general the temperature sequence is much more stable from year to year than the precipitation sequence. It has already been mentioned that the annual temperature coefficients are for the most part distributed at least approximately in accordance with the normal law of frequency about the mean values characteristic of each station. The standard deviation of corresponding coefficients $b' \dots f'$, shown at the foot of Tables I, II, and III, is very similar at all locations. That of a' is greatest at Swift Current (1.82°) and least at Edmonton (1.43°), but this difference likewise is statistically insignificant. Furthermore, there is a considerable degree of association between the annual fluctuations at the three stations of each of the coefficients $a' \dots d'$ investigated in Table IV, which accounts for from 73 (d') to 84% (b') of the total annual variance. On the other hand no significant correlation in the variation from year to year of the mean temperature for the season (a') and that of any of the sequence coefficients $b' \dots f'$ was demonstrable at either Winnipeg, Swift Current, or Edmonton, indicating that in general the phase and amplitude of the temperature sequence are the same in seasons of below-average mean temperature as in those above-average. This is in contrast to the situation found to prevail in respect of precipitation (3).

As in the case of the rainfall coefficients previously considered, the secular trend of the temperature coefficients for each station was investigated by a further regression analysis in which a fifth degree polynomial function of time was fitted to each series of 44 $a' \dots f'$, also by the use of Fisher and Yates' multipliers (2). With one exception the resulting regression coefficients proved to be uniformly negative, leading to the deduction of an essentially random incidence, without orderly sequence in time, of the annual variations described above. The exception mentioned is provided by the quadratic coefficient c' for Edmonton which, as illustrated in the upper part of Fig. 2, has shown a slight trend over the period of record (accounting for about 30% of the annual variance) resolvable into components tentatively ascribable to a long-term oscillation, of about 30-yr. phase, superimposed on a gradual progressive

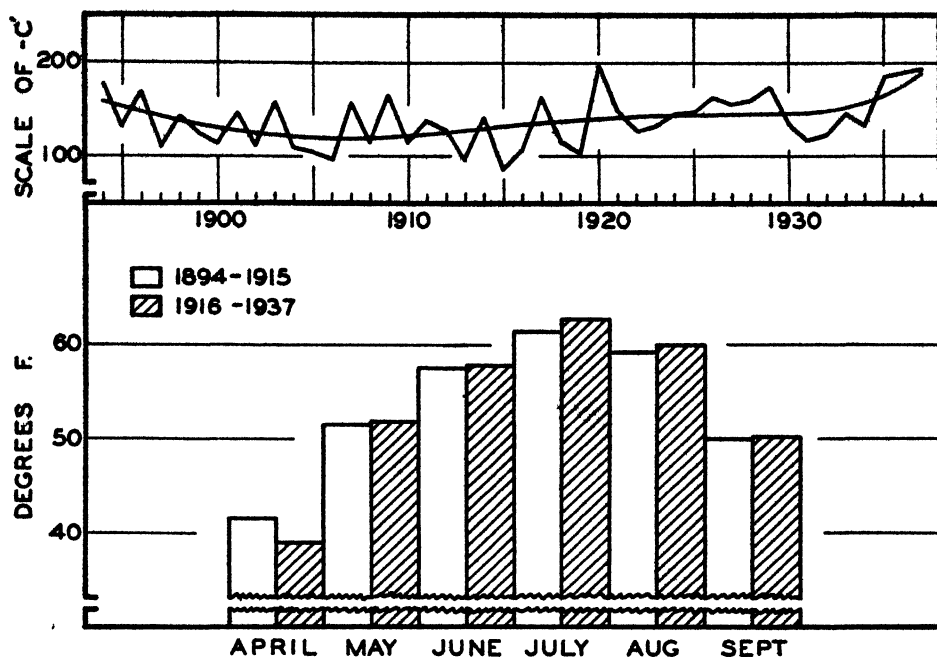


FIG. 2. Upper portion: Annual fluctuations and secular trend of the temperature coefficient c' for Edmonton, 1894-1937. Lower portion: Average summer sequence of monthly mean temperature at Edmonton, 1894-1915 and 1916-1937.

increase. This is reflected in the monthly mean temperatures for the periods 1894-1915 and 1916-1937, the average for April being 2.5°F. lower in the latter, whilst the averages for July and August are respectively 1.2 and 0.8° higher. In this connection, it may be remarked that Edmonton was likewise the only one of these three stations to show any progressive change in the seasonal incidence of precipitation (3).

TABLE V

COVARIANCE IN ANNUAL FLUCTUATIONS OF TEMPERATURE AND RAINFALL COEFFICIENTS

Sequence coefficient	Coefficient of correlation		
	Winnipeg	Swift Current	Edmonton
a'	+0.01	-0.33*	-0.23
b'	-0.01	-0.23	-0.08
c'	-0.05	-0.28	-0.12
d'	-0.01	-0.36*	-0.23
e'	-0.16	-0.35*	-0.32*
f'	-0.08	-0.16	+0.05

* Exceeds 5% level of significance ($r = \pm 0.30$).

Covariance of Temperature and Rainfall Coefficients

Covariance in the annual fluctuations of corresponding rainfall and temperature coefficients was investigated by calculation of the correlation coefficients listed in Table V. Sixteen of the 18 are negative in sign, and four may be regarded as individually statistically significant. There is thus some suggestion of an inverse relation between seasonal temperature and precipitation, at Swift Current and Edmonton at any rate, but the indications are that for the most part this is rather tenuous.

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THE ORIGIN AND HISTOLOGY OF BORDEAUX SPRAY RUSSETING ON THE APPLE¹

BY HUGH P. BELL²

Abstract

Apple trees of the McIntosh Red variety were sprayed at about the time of full bloom in 1939 and 1940. The origin and structure of the resultant russet tissue is described. The first apparent injury is a browning of the epidermal cells at the base of the hairs. The growth of these browned cells is inhibited and, owing to this, cracks occur as the fruit enlarges. Adjacent hypodermal and cortical tissue is exposed and killed. Cork cambiums and cork are formed in the cortex. This cork is different in origin from normal russet cork, which originates in the epidermis. The further enlargement of the fruit causes the cracks to multiply, extend tangentially, and deepen. All tissues external to the innermost point of fissure penetration become killed. The final scurf-like patches of scar tissue are a mixture of dead epidermis, hypodermis, cortex, cork, and cork cambiums. This scar tissue is not true cork.

Introduction

As there is an appreciable annual loss of apples from russetting induced by spray materials, it was considered advisable to obtain information regarding the origin and histology of the russet tissue appearing on fruit sprayed with Bordeaux mixture under field conditions. The investigation reported below was undertaken to provide this information.

Preliminary Studies

The morphology of abnormal tissue can be studied most effectively by comparing it with normal tissue. With this in mind, the development of the protective layers of both the McIntosh Red (2) and the Golden Russet (3) were worked out in detail. These normal forms of development were used as a basis for comparison in this study of the abnormal tissue of induced russetting.

Material and Technique

The material for the study was obtained from trees of the McIntosh Red variety, at the Dominion Experimental Station, Kentville, N.S. The trees were sprayed with Bordeaux mixture as follows: in 1939, on May 31 and again on June 7; in 1940, on May 22 and again on June 11. The date of full bloom for the McIntosh Red at Kentville in 1939 was June 8 and in 1940, June 4. In both years this treatment provided an ample supply of russeted fruit.

From the day the spray was applied, the material was collected twice each week in the early part of the season and once a week during the latter part of the season. This material was treated in two ways. Part was killed in

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Contribution from the Department of Biology, Dalhousie University, Halifax, N. S., with financial assistance from the National Research Council of Canada.

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chromacetic, imbedded in paraffin, and examined in the form of serial sections that had been stained with safranin and fast green. The other part of the material was cut while still fresh and the free-hand sections were mounted in glycerine jelly. For the identification of the injured tissues, especially the early stages, the free-hand sections mounted in glycerine jelly were the more valuable for in these the original brown discoloration could be seen in sharp contrast to the green or colourless normal cells. To detect the very early stages, tangential sections are the best. The most satisfactory way to obtain these is to use a sharp safety razor blade and do the actual cutting under the lower power lenses of a binocular dissecting microscope. In this way a section can be obtained that contains little more than the epidermal layer.

Development and Histology

During the period immediately following the application of the spray, a large amount of material was examined very carefully, but injury to the tissues was not observed until from 10 to 15 days after the first application. This agrees with the findings of Young and Walton (9, p. 412) who state "a long period may elapse after the application of a copper spray before the injury appears". When the first signs do appear they develop almost simultaneously on a large percentage of the young fruit. This first indication of an injury is a conspicuous browning of the epidermal cells and is quite easily identified by means of the tangential sections of fresh material. At first this browning occurs only in the epidermal cells immediately surrounding the hair base (Figs. 1 and 3), but very shortly it spreads to the adjacent epidermal cells (Fig. 4) and to the hypodermal layer immediately below the epidermis. Almost immediately a series of tangential divisions occur in the hypodermal cells beneath the hair. This elevates the injured tissue slightly above the remainder of the epidermis (Fig. 2). Except for the fact that these divisions occur in the hypodermis and not in the epidermis, they are similar to those that normally precede the formation of a cork cambium in the Golden Russet apple (3, p. 564, Figs. 10, 11), but these cambium initials below the injured tissue form merely patches of cork and these are seldom more than three or four cells in thickness, for, as will be described later, the cambium cells are almost immediately inactivated by the further progress of the injury.

Occasionally a similar browning may be observed in the cells surrounding a stoma (Fig. 5), but in most cases the guard cells and the adjacent epidermal cells are unaffected. When they are browned, the progress of the injury is similar to that which has been and will be described for the injury originating at the hair base.

At the time browning first appears, there is some evidence that the application of the spray may stimulate the activities of some epidermal cells and more definite evidence that it inhibits the activities of others. The apparent *stimulated activity* occurs in some of the epidermal cells adjacent to, but not in, the injured area and consists of a single tangential division (Fig. 9). This is exactly similar to the first division in the formation of a normal periderm in

the Golden Russet apple (3, pp. 561 and 562, Figs. 1 to 6). In the McIntosh Red fruit injured by Bordeaux spray, this activity does not proceed beyond the formation of one tangential wall. Such tangential division of the epidermal cells has not been observed by the author in the normal development of the McIntosh Red. This of course is only negative evidence and the number of observations that can be made by any one investigator is limited; also no controlled experimental work regarding the appearance of the wall was attempted, so there is no proof that stimulation occurred; but, in the material examined from both normal and sprayed fruit, the evidence as outlined above appeared to suggest some association between the application of the spray and the formation of this single tangential wall. The *inhibition of normal activity* occurs in the epidermal cells that are browned. To see this, an injury at the stage illustrated in Fig. 2 must be examined in a stained microtome section. In such a mount the cell contents are not masked by browning as they are in the hand sections mounted in glycerine jelly, consequently it can be seen that the affected epidermal cells have normal nuclei, unplasmodysed cytoplasm, and a cuticle that has thickened uniformly over the whole surface. These cells are usually conspicuous, owing to their raised position, but whether they are or are not raised, they may be identified by their shape, for in their development they have not kept pace with the unaffected epidermal cells. These go through a regular sequence of rapid changes in shape and size as the fruit starts to enlarge (2, p. 396), but during the two weeks subsequent to the application of the spray the affected or browned cells remain unchanged so far as shape and size are concerned. This suggests that the normal growth of the affected cells was inhibited about the time that the spray was first applied. Thus the application of Bordeaux spray about the time of full bloom may have some relation to the appearance of a tangential wall in certain epidermal cells and it apparently inhibits the normal development of other epidermal cells, namely, those that are turned brown.

A few days after the first appearance of the injury, cracks develop on the surface of the young fruit. These usually appear at about the centre of the browned areas and after extending inward through the epidermis and dividing hypodermal cells, they expose what had, up till then, been unaffected cortical cells (Figs. 6, 7). These cortical cells immediately turn brown and at the same time the browning effect spreads to all the cells in the immediate vicinity. The whole lesion in cross section is now a lens-shaped mass of browned tissue with the centre of its inner convex surface at the point to which the fissure penetrates most deeply (Fig. 8). During the inward progress of the browning, the cork cambium initials, which had been formed beneath the hair base, collapse and become indistinguishable. The injured epidermal, hypodermal, and cortical cells turn a very dark brown and collapse. The disintegrated cell contents are deposited on the cell walls, giving to these walls the appearance of being very thick. Again, and in limited patches just inside the lesion, the initial stages of a secondary cambium frequently develop (Fig. 9), but sometimes there is no evidence of such cambium formation

(Fig. 8). In the latter case many of the cortical cells immediately inside the lesion may elongate radially or some of the large cortical cells may divide by a tangential wall (Fig. 7), but these tangentially divided cortical cells are not necessarily arranged in any definite layer. They may be scattered at random among the cells just inside the injury. These secondary activities of both cambium formation and tissue proliferation are early inhibited for the cells involved are very soon both exposed and isolated as a result of the still further inward penetration of the fissure.

The subsequent development of the injury is probably brought about by the enlargement of the fruit and there is a continuous repetition of the process described above. That is, the fissures deepen, the browning effect extends to all adjacent cells, and cork cambium formation and other induced activities are initiated, but almost immediately inhibited by the continued deepening of the fissures. While the injury is penetrating into the cortex, the cracks lengthen rapidly in a tangential direction on the surface. Also new cracks develop in the browned tissue on each side of the original ruptures. These various splits criss-cross like a fine network over the surface of the apple. The result is that, in the mature fruit, the injury consists of many small, medium, and large cracks, which are separated by browned, dried masses of epidermal, hypodermal, and cortical tissues mixed with scattered patches of cork (Fig. 10). It is these brown scurf-like masses of dead cells that give to the injury its russeted appearance. Russet tissue of this type may be found during late June or early July and except for becoming thicker and more extensive, it does not change during the subsequent development of the fruit.

The mixed composition of this tissue is brought out clearly by micro-chemical tests. The patches of cork give the reactions typical for suberin. Enclosed between these cork layers, there are groups and layers of dead parenchyma cells, the walls of which give the typical cellulose test. There are also many cells that are parenchyma-like in shape, but with walls that react for neither suberin nor cellulose. The exact chemical composition of the walls of these cells was not determined. A cuticle, associated with collapsed epidermal cells, still adheres in places. When present it is easily differentiated by the usual tests. Thus, by this means, four or five different types of tissue may be identified in the lesion.

The method by which the spray penetrates the cell was not determined, but observations were made that furnish negative information. Attention was directed to this problem by Horsfall and Harrison (5, p. 441) in their paper dealing with Bordeaux injury to the tomato. They put forward the theory that the spray "saponifies the cuticle". Having this statement in mind all material was examined very carefully to see if any evidence could be found to suggest that this theory was applicable to Bordeaux injury on the apple. It is not difficult to make observations on this point for, at the time the apple is in full bloom, the cuticle over the cells immediately surrounding the hair base is slightly thicker than that over the average epidermal

cell (2, p. 394, Fig. 4), thus it is quite easily seen in hand sections of fresh material. All the sections studied indicate that at the time the injury first appears the cuticle is still intact, it is of normal thickness, and it does not show any sign of having been corroded in any way. That is, there is no morphological evidence suggesting saponification of the apple cuticle by Bordeaux spray.

Discussion

This russetting induced by Bordeaux spray is different in both origin and structure from that developed as a normal healthy growth on such varieties as the Golden Russet. The origin of the secondary cambium of normal russetting is in the epidermis (3), but in induced russetting, the secondary cambium originates in the hypodermis or cortex. In structure, normal russetting is a homogeneous cork tissue, but induced russetting is a mixture of dead epidermal, hypodermal, and cortical cells plus patches of cork.

From a histological standpoint it is not correct to use the word "cork" to designate this scar tissue of induced russetting. It is more like *rhytidome*, but it has been pointed out that one could not use this term instead of cork since *rhytidome*, as defined by Eames and MacDaniels (4, p. 212), connotes alternate layering of cork and dead cortical or phloem tissue, whereas the scar tissue of the sprayed McIntosh Red apples is a *mixture* wherein there may not be any cork formation. In an exact botanical description it would be best to call it merely "scar tissue". Of course in popular phraseology the term "cork" is so well established as descriptive of this tissue that it will probably always be used in papers that are not purely scientific and histological.

In the literature there are a number of descriptions of induced russet tissue on apples and in these descriptions most of the investigators refer to this russet tissue as "cork". For instance, Baker (1, p. 78) in his study of this injury states:—"This russetting consists of a corky covering to take the place of the normal cuticle and epidermis". Also Verner (8, p. 817), in discussing what is apparently a similar lesion, describes it as follows:—"A layer of cork cambium assumes the position normally occupied by the epidermis, cutting off to the outside several, or many, tangential layers of cork cells, which constitute the scurflike russet". In a paper by MacDaniels and Heinicke (6) there is a record of russet tissue that is altogether cork. They consider that frost was the cause of the injury and they both describe and figure the lesion as a typical periderm. In a case of russetting observed by Tetley (7, p. 165), she describes the tissue as "several layers of dead cells" and goes on to state that these are "above a continuous layer of cork and the meristem layer from which it is derived". The first part of her description (but not the latter part regarding a continuous cork and meristem layer) would be applicable to the Bordeaux injury under investigation. According to the statements of these and other investigators, induced russet tissue on apples may at times be only true cork, and hence quite different from that produced by Bordeaux spray on the McIntosh Red.

Why do the initial breaks in the surface occur wherever the cells have been browned by Bordeaux? The answer to this question is of importance for, after the original fissures are formed, the later stages of the injury follow automatically with the enlargement of the fruit. It cannot be said that these discoloured cells are immediately killed, for as stated above they still have a normal nucleus, an unplasmolysed cytoplasm, and a cuticle that continues to thicken; according to Verner (8, p. 820), however, the death of the epidermal cells would not be a necessary prelude to the formation of a crack. His statement on the subject is as follows:—"the problem of cracking in the apple involves inability of the peripheral region to stretch or grow as rapidly as it should when the fleshy portion of the fruit is enlarging at an abnormal rate". That is, the inhibition of tangential growth at the surface would be all that is necessary to cause fissures to form through the surface layers. From the evidence submitted above, it is apparent that such an inhibition does occur in the browned epidermal cells. This being the case, the epidermis cracks because the Bordeaux spray has inhibited its normal growth.

Acknowledgments

The author wishes to express his indebtedness to the Pathologist-in-Charge, Laboratory of Plant Pathology, Kentville, N.S., for laboratory space during two summers. The figures were drawn by Miss Elizabeth E. Bligh of Kentville.

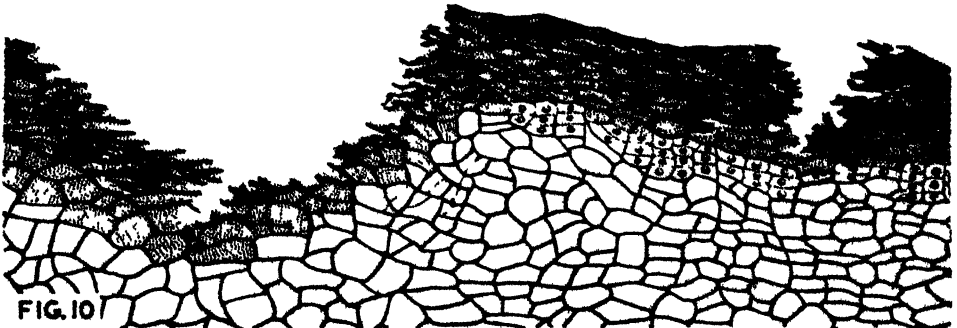
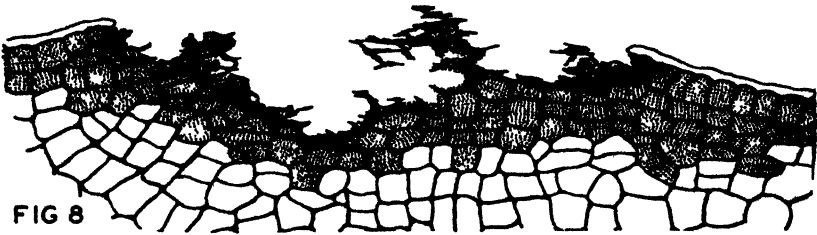
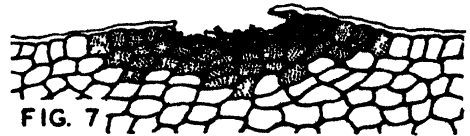
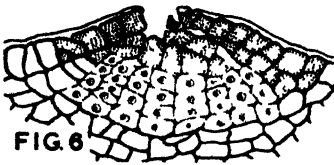
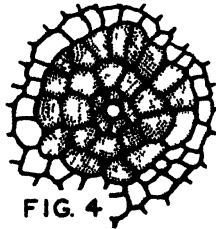
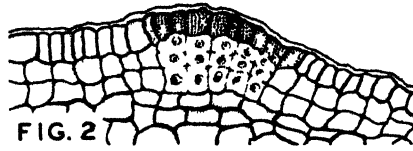
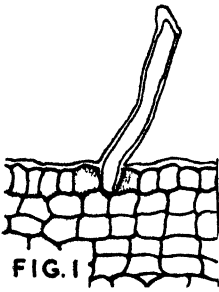
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EXPLANATION OF FIGURES

Magnification: Figs. 1 to 9, 240 \times ; Fig. 10, 130 \times .

FIG. 1. June 12. Section through a hair base with the browned epidermal cells at the base of the hair. FIG. 2. June 12. Section of browned area before the epidermis has ruptured. A secondary cambium is formed. FIG. 3. June 17. Surface view of the stage illustrated in Fig. 1. FIG. 4. June 27. Surface view of the stage illustrated in Fig. 2. FIG. 5. July 2. Surface view of guard cells and adjacent epidermal cells turned brown by Bordeaux spray. FIG. 6. June 27. Section of browned cells just after the surface has ruptured. FIG. 7. June 24. Section of a stage slightly more advanced than that illustrated in Fig. 6. The fissure has broadened and the first formed cork cambium cells have died and collapsed. The radial arrangement of the cortical cells suggests tangential division in that tissue. FIG. 8. July 2. Section of a fairly advanced stage in which no secondary cambium is evident. Some of the cortical cells are elongated and radially arranged. FIG. 9. June 28. Section of a fairly advanced stage in which a definite secondary cambium has been formed. Two epidermal cells on the left of the section have divided by a tangential wall. FIG. 10. July 4. Section of a mature lesion. The fissure on the left is penetrating into the cortex in advance of cambium formation. The fissure on the right is just penetrating through a cork cambium. The outer tissue of the lesion is so collapsed and crushed that its cell structure is indistinguishable.



STUDIES OF THE TOMATO IN RELATION TO ITS STORAGE

II. THE EFFECTS OF ALTERED INTERNAL ATMOSPHERE UPON THE RESPIRATORY AND RIPENING BEHAVIOUR OF TOMATO FRUITS STORED AT 12.5° C.¹

BY K. A. CLENDENNING²

Abstract

When a mature green tomato fruit is stored at 12.5° C. either with or without its stem, the expected respiratory climacteric accompanies the colour change associated with ripening. When the stem is removed and the stem scar area is covered carefully with hot paraffin wax, the fruit thereafter ripens slowly with a low, relatively constant rate of carbon dioxide output. These characteristics are ascribed to "auto-gas" storage resulting from restricted diffusion at the stem scar. The effect of waxing is reversible within limits since removal of the artificial seal after one month has resulted in a return to normal ripening and respiratory behaviour.

When yellowing, yellow orange, and full red fruits are stored either with or without their stems, they complete in storage those phases of the respiratory climacteric that had not been completed before detachment from the plant. The careful waxing of fruits picked at these stages of maturity inhibits further coloration and reduces the rate of carbon dioxide output to the same extent as in fruits waxed at the mature green stage. The respiratory drift of fruits picked and stored unwaxed at the early "growing green" stage is characterized by two distinct peaks. Such fruits eventually ripen and the second peak is associated with the colour change that accompanies ripening. Similar fruits stored with stem scars waxed fail to ripen before their pathological "death" and their respiration rate is reduced by the waxing treatment.

When yellowing and yellow-orange fruits are waxed, they become soft and highly susceptible to fungal attack before their ripening coloration has been completed. To inhibit the softening process in stored tomatoes, it thus appears to be necessary to apply wax before ripening has commenced. Unwaxed fruits become highly susceptible to fungal wastage only after attaining full ripeness. Waxed fruits on the other hand are subject to fungal wastage when green or partially coloured as well as when fully ripened. This is attributed to the progress of softening in the absence of the usual colour change associated with ripening. Waxing of the stem scars does not act as a deterrent to storage moulds at the waxed area. The waxed tomato has been found to be subject to several physiological disorders the symptoms of which are described.

Walford (16) observed two physiological types of tomatoes when fruits, picked at the "mature green" stage, were stored at 12.5° C. The fruits of late spring and summer showed a distinct rise in respiration rate as they ripened in storage while the late autumn and winter fruits exhibited a marked durability, colouring slowly and unevenly without an attendant rise in carbon dioxide output. He designated these contrasting types as "conventional" and "anomalous", respectively, and provisionally concluded that their distribution was related to seasonal factors.

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Contribution from the Department of Horticulture, Ontario Agricultural College, Guelph, Ont., with the co-operation of the Department of Botany, University of Toronto, Toronto, Ont. This paper constitutes part of a thesis submitted to the Graduate School of the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Issued as Paper No. 72 of the Canadian Committee on Storage and Transport of Food.

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At this laboratory, information has been accumulated on the distribution of these divergent types in relation to seasonal and experimental light conditions. Both types have been found at all seasons of the year as well as under different light intensities in the same season. Also, intermediate types appeared sporadically and they, too, had no apparent association with any special growing conditions. The preponderance of anomalous types during the winter season or under simulated winter light conditions and of conventional types during the summer in certain phases of this work suggested that, as Walford supposed, the seasonal light factor might influence the distribution of types actually present at all seasons. The complete absence of confirmatory evidence in bulk storage trials, however, demonstrated that the seasonal influence, if operating at all, was overshadowed by other determinants.

Singh and Mathur (15) reporting on the tomato fruit in relation to storage temperature stated that some of their fruits, when picked at the mature green stage passed through the ripening colour change in storage without the usual rise in respiration rate. The record submitted as typical of these was obtained at 5.7° C.; it exhibited the low, steady respiration rate and slow colour changes that characterized Walford's anomalous type.

The anomalous type is not limited to preclimacteric picks since fruits detached from the vine at the yellow and yellow orange stage frequently have fallen into this category. And further, Walford submitted records of "early growing green" fruits that showed a distinct climacteric when the colour changes occurred. The conventional type therefore was by no means restricted to fruits picked at late, and the anomalous type to fruits picked at early stages of maturity.

Examination of unpublished data available at this laboratory showed that the solution of the problem did not lie in the field of genetics since conventional and anomalous types had been obtained from the same plant. Similarly, the presence of both anomalous and conventional types within visibly uniform populations of plants or in the product of a single plant would indicate that nutrition is not the determinant of physiological type.

Nevertheless, the possibility of a relation to certain aspects of mineral nutrition has been explored incidentally by co-workers in this laboratory. The author is indebted to them for permission to refer to their results. Populations of plants were grown in parallel series receiving different amounts of nitrogen, phosphorus, and potassium, and supplied with various kinds and amounts of organic matter. Concurrent soil tests and the appearance of the plants showed that the populations were actually receiving different amounts of the nutrients named. Under all treatments the anomalous type tended to predominate with conventional types appearing only occasionally.

Phillips (13) has drawn attention to certain differences in the respiration records of fruits grown under three boron treatments. Under Walford's classification his "low boron" fruit would be designated conventional. The

"excess boron" fruit evidently was picked after the senescent rise in respiration but the pitch of its respiratory activity and its early breakdown would place it in the same category. Assuming that his "medium boron" fruit ripened in the recorded storage period it would be classed as intermediate since its respiration rate is higher than our arbitrary upper limit for anomalous records. On the basis of these observations Phillips concluded that medium boron treatment had a steadying effect on carbon dioxide output and that appropriate boron administration would lead to a low respiration rate conducive to better keeping properties. However, the diversity of types recorded by him in relation to boron nutrition is not as great as the range found in more extended investigations employing populations receiving a constant fertilizer treatment (15, 16). From this it must again be concluded that mineral nutrition is not of primary importance in the production of the physiological states of the tomato fruit with which this paper deals.

The present paper includes a survey of the relation of physiological types to season and to the internal atmosphere of the fruit as altered by waxing the stem scar. Finally, undesirable accompaniments of waxing are discussed with relation to the possible commercial application of this technique.

Materials and Methods

The populations of *Lycopersicon esculentum* Mill. var. Grand Rapids employed in this work were grown in summer outdoor plots, both shaded and unshaded, and, by accepted commercial methods (16), in greenhouse benches at all seasons of the year.

The fruits were detached from the vine at the stages of maturity indicated. After removal of the stems, the fruits were weighed individually and stored at 12.5° C. within two hours of harvesting. Fruits stored in trays were well ventilated and the relative humidity of the storage atmosphere was held between 80 and 85%. Fruits stored in respiration chambers were subjected to a steady stream of carbon dioxide free air whose relative humidity was maintained at approximately 85% by bubbling through 18% potassium hydroxide. Measurements of carbon dioxide output were made by the usual Pettenkofer method employing absorption periods of 24 hr.

Walford found that moulds developed quickly on unprotected stem scars under conditions of high relative humidity. To overcome this, he applied wax to the stem scars as this seemed to offer some protection. This practice had not been followed in earlier investigations (1, 7) in which only conventional respiration types were observed. In subsequent work (13, 15, 16) in which the stem scars were covered with wax, anomalous respiration types occasionally appeared. In the earlier part of the present investigation the stem scars were covered with wax. Subsequently the waxing was deliberately varied to extremes as noted in the text.

Results

The Distribution of Physiological Types in Relation to Season

During the year 1938 a survey of the effect of season on the distribution of physiological types was carried out by studying the respiration of individual fruits picked at the mature green stage in January and June. It was expected from Walford's work that the January fruits would be all anomalous and the June fruits all conventional. Actually the January fruits were all conventional and the June fruits gave a mixture of anomalous, conventional, and intermediate types.

The Distribution of Physiological Types in Relation to Light Treatment

In 1939 a comparison was made of the types of respiration exhibited by fruits picked at the mature green stage from summer populations grown simultaneously under three light treatments using (a) greenhouse benches under ordinary window glass, (b) a bench out-of-doors unshaded, (c) a similar outdoor bench shaded with cloth to 50% light intensity.

As is shown in Table I, the fruits of these three plots were a mixture of physiological types—anomalous, conventional, and intermediate records having been obtained from each population. Further, the anomalous type predominated in each plot, the conventional and intermediate types appearing sporadically.

TABLE I

THE DISTRIBUTION OF PHYSIOLOGICAL TYPES IN RELATION TO LIGHT TREATMENT

Plot	Physiological type		
	Anomalous	Conventional	Intermediate
Summer greenhouse, 1939	10	3	3
Outdoor unshaded, 1939	9	2	2
Outdoor shaded to 50% light intensity, 1939	12	1	1

Physiological Types in Relation to the Artificial Stem Scar "Seal"

Brooks (3) maintains that the gaseous exchange of the tomato fruit is principally through its stem scar; he attributes this to the relatively impervious nature of the fruit at other parts of its surface. He finds that restriction of gaseous interchange at the stem scar has an unmistakable effect on the time required for ripening. Waxing the stem scar in itself doubles the time that mature green fruits can be held at 70° C. before ripening occurs. On the other hand, application of wax to the remaining surface of the fruit with the stem scar left unsealed has little effect on the rate of coloration. He supplements these interesting observations with measurements of the carbon dioxide content of the internal atmosphere. Fruits stored with stems attached have a measurably higher carbon dioxide content than fruits with the stems removed

and they have a slower ripening rate. He also shows that a marked alteration of the internal atmosphere of the fruit results from waxing the stem scar, the concentration of carbon dioxide within the fruits so treated being twice that of the checks, over a wide range of temperatures.

Retardation of the ripening process by restriction of gaseous diffusion at the stem scar has been observed at other laboratories. Wardlaw and McGuire (17) have noted that fruits stored with stems attached ripen more slowly than similar fruits with stems removed. At this laboratory it has been found that waxing the stem scar is much more efficient in retarding the ripening process than is the practice of merely allowing the stems to remain.

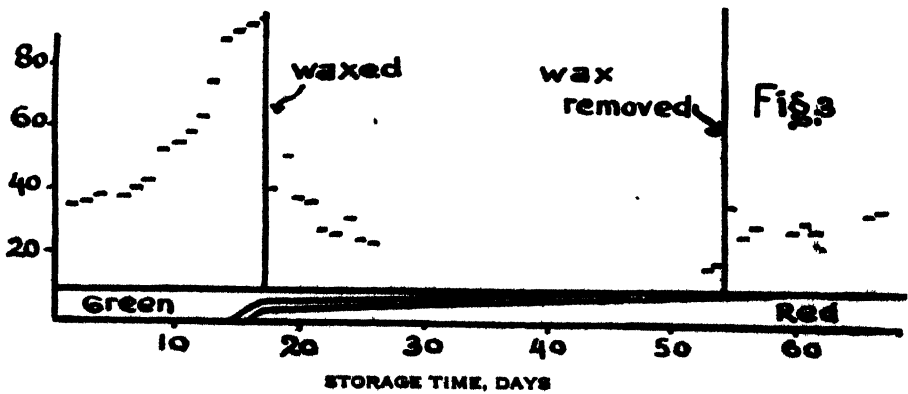
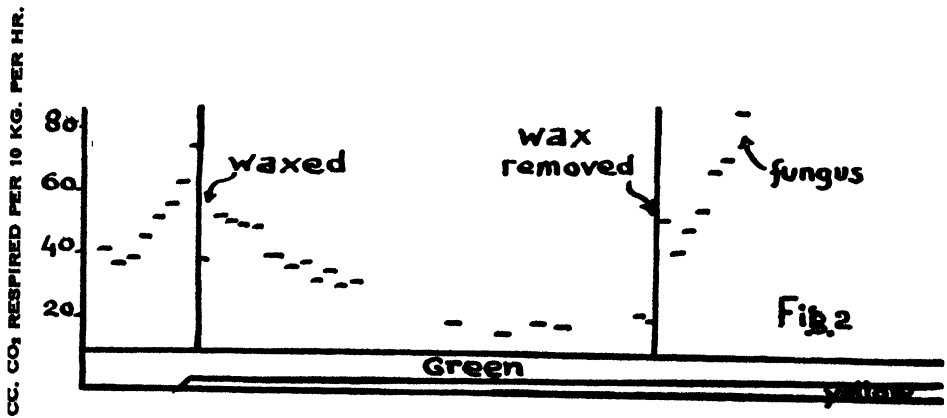
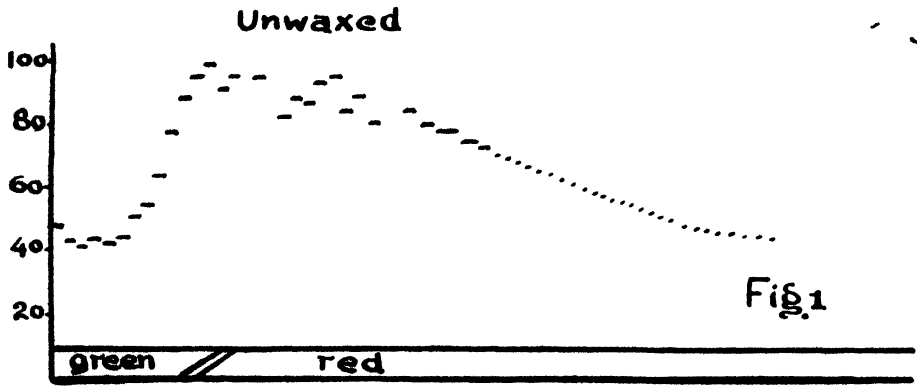
If these alterations of rate of ripening are to be attributed to altered internal carbon dioxide and oxygen concentration, the production of similar internal atmospheres by other means should have a parallel effect. Kidd and West (10) have stored tomatoes in artificial gas mixtures and they report that the colour change associated with ripening is retarded by both a decrease in oxygen and an increase in carbon dioxide of the storage atmosphere. Gustafson (8) offers further evidence on this point in finding that tomatoes stored in air ripen more rapidly than similar fruits kept in sealed containers.

From this it would appear that any storage practice causing a marked increase in the carbon dioxide or decrease in the oxygen content of the internal atmosphere of the tomato fruit will lead also to the slow ripening rate that so constantly accompanies the anomalous type of respiration. In the work reported here, the respiration of mature green fruits has been studied in the waxed and unwaxed condition; 18 fruits were stored in individual respiration chambers with stem scars sealed carefully by a copious application of hot paraffin wax and 24 fruits were stored with stem scars untreated. *In every instance the carefully waxed fruits were of the anomalous type whereas the unwaxed fruits were always conventional in their ripening and respiratory behaviour* (Figs. 1, 5).

In earlier respiration studies in which the anomalous type has appeared (13, 15, 16), waxing the stem scar was practised as a matter of routine. Evidently no particular care was taken to control rigorously the effectiveness of the waxing. As a result a mixture of physiological types appeared among the waxed fruits.

The cases of greatest interest were those in which a serious restriction of gaseous interchange at the stem scar resulted from the waxing treatment. Undesirable accompaniments were usual and these are discussed in detail below. However, in some cases the sealing of the stem scar with wax has occasioned a highly desirable "auto-gas storage" of the fruit, the fruit itself inducing by its own respiration those changes in internal carbon dioxide and oxygen tension that lead to a greatly enhanced storage life.

In some instances the wax cover must have resulted in a partial seal at the stem scar so that the respiration and ripening rate was inhibited to some extent. The occurrence of the intermediate type is attributed to such a partial success in sealing.



FIGS. 1 TO 3. Effect of waxing on respiration rate of mature green tomato fruits in storage.

Occasionally, placing a drop of wax on the stem scar had little effect on gaseous exchange at this area, doubtless because a "seal" was not effected. This could be expected fairly consistently where, for instance, the wax was at a relatively low temperature at the time of application. As a result the "conventional" characteristics would be observed.

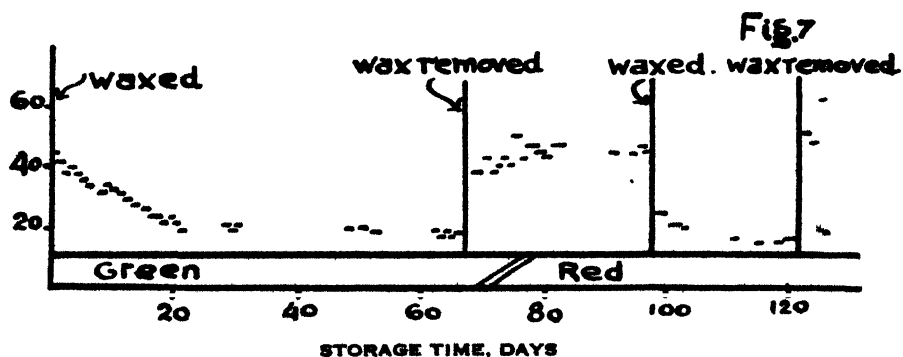
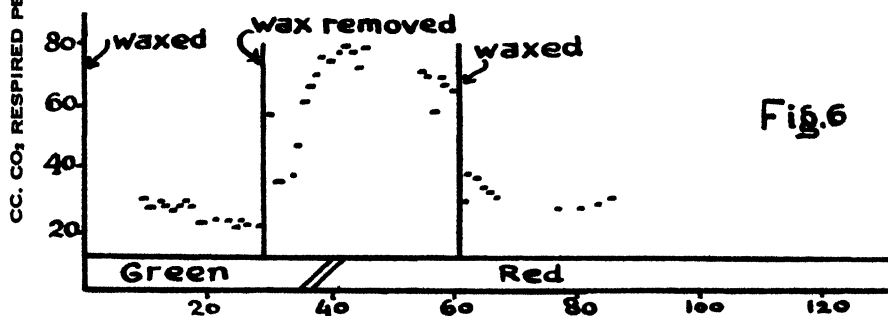
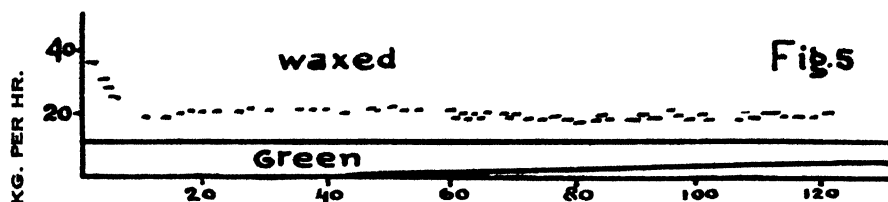
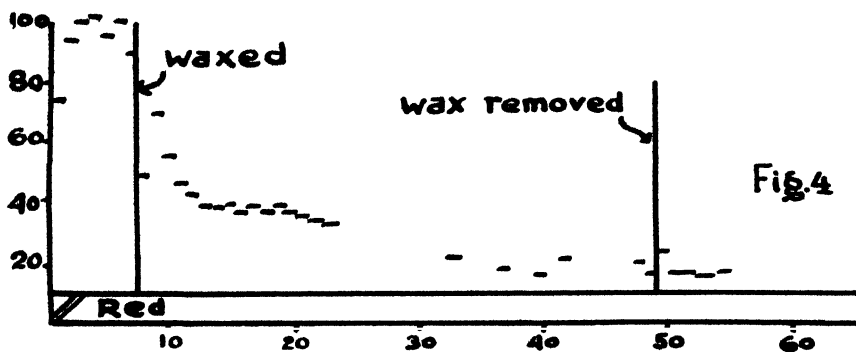
Ascribing the anomalous or auto-gas stored type to altered internal atmosphere, the question next arises as to the feasibility of changing the anomalous to the conventional type simply by removing the wax from the stem scar area. Accordingly fruits were picked at the mature green stage, the pedicels were removed, and the fresh weight of the fruit recorded. The stem scar areas then were filled with hot paraffin wax. The fruits were stored immediately in respiration chambers at 12.5° C. and their respiratory rate followed (Figs. 6, 7, 8). The respiration rate fell to low steady values because of the restriction of gaseous traffic at the stem scar area. After different periods in the "sealed" state the wax was removed and the respiration measurements continued. The fruits from which the wax was removed after about one month's storage then showed the ripening and respiratory behaviour that characterizes the conventional type. When the respiration in this "unsealed" state was declining slowly but steadily from the peak observed in the midst of the colour changes, the stem scar area was once again waxed copiously with paraffin. Thereupon the respiration rate decreased to the same level as was observed before the wax was removed.

In one instance (Fig. 6) the rate of carbon dioxide output of a carefully sealed fruit was followed for one month. Then, before there was any evidence of external ripening coloration, the wax was removed. The high value observed immediately after wax removal is attributed to the liberation of accumulated carbon dioxide. Thereafter the carbon dioxide output is taken to be truly indicative of the respiratory activity of the fruit. A typical senescent rise in respiration was observed as the fruit ripened. Thirty-one days after wax removal, wax was once again applied carefully to the stem scar and thereupon the respiration of the fruit declined to the level observed before the wax was first removed.

Fig. 7 is typical of the records obtained when the wax was allowed to remain for two months or more during which time the fruits ripened partially. Wax removal in these cases resulted in an increased rate of carbon dioxide production but the rate after wax removal was lower than in fruits from which the wax was removed after relatively short storage periods.

In order to establish the effects of waxing the stem scars of fruits picked at the mature green stage and stored for some time before wax application, the following experiment was conducted. The rate of respiration was followed in a series of such fruits stored in the unwaxed condition. Then, when various stages of ripeness had been attained, wax was applied carefully to the stem scars; Figs. 2, 3, and 4 are typical of the records obtained.

Fig. 2 shows the typical respiratory changes observed when wax was applied just as the coloration associated with ripening was becoming externally visible.



FIGS. 4 TO 7. Effect of waxing on respiration rate of mature green tomato fruits in storage.

The results of wax application at the peak of the respiratory climacteric is shown in Fig. 3. Wax also was applied to fruits that had ripened completely in storage; the accompanying respiratory changes are shown in Fig. 4.

These figures show that fruits of the conventional type can be made to exhibit all the respiratory characteristics of Walford's anomalous type simply by sealing the fruit at its stem scar after any stage of ripeness has been attained in storage.

After periods of one month or more in the "sealed" condition, the wax was removed from the stem scars of these fruits. Usually wax removal resulted in a reversion to respiration rates of the order found in unwaxed fruits stored for a similar length of time. However, divergences from this did occur and Figs. 2 and 4 represent the extremes observed.

The transitional changes in carbon dioxide output, shown immediately after wax application and again on its removal, have been consistent features of these waxing experiments in all but a few instances. For example, in Fig. 6, immediately after wax removal the initial reading is high because of the liberation of accumulated carbon dioxide but this complication is evidently absent after the first day. Thereafter the carbon dioxide output is truly indicative of the respiratory activity of the fruit.

On applying wax to the stem scar a month later, the first respiration reading was low because of the resulting restriction of carbon dioxide emission at the stem scar area. The succeeding values were higher because of the mounting internal carbon dioxide concentration and hence increasing diffusion through the skin. The rate of emission then tended to fall, through the depressing effect of accumulated carbon dioxide on the respiratory mechanism.

Thus on waxing the stem scar of the tomato fruit there is first, a period of rapid carbon dioxide accumulation within the fruit and a low rate of emission; secondly, a period in which internal carbon dioxide content is high and rate of both carbon dioxide production and emission falls off; finally, there is an equilibrated phase in which the rate of carbon dioxide emission is almost if not completely constant.

The final stage must be associated with a lower internal carbon dioxide concentration than exists in the second phase if the rate of gas emission is a function of its internal tension. In this connection Claypool (6) found the carbon dioxide content of completely waxed fruits to be high initially and to decrease during prolonged storage.

These changes in ripening and respiratory behaviour, as induced by wax application and wax removal, are attributed to the accompanying changes in the internal atmosphere of the fruit. This is supported by analyses of extracted gas samples from fruits stored for short periods with and without wax on the stem scars (Table II).

In making these determinations the extraction method of Brooks (2) was followed. Carbon dioxide and oxygen estimations were made on the standard Haldane gas analysis apparatus after suitable dilution with nitrogen.

TABLE II

THE EFFECT OF WAXING ON THE COMPOSITION OF THE INTERNAL ATMOSPHERE OF THE TOMATO FRUIT

Treatment	Storage time, days	Maturity of fruit	Air extracted in vacuum, 3 min.	
			CO ₂ , %	O ₂ , %
Unwaxed throughout	1	Mature green	7.0	19.3
Unwaxed throughout	8	Mature green	7.45	23.6
Unwaxed throughout	9	Colouring	12.35	19.2
Waxed initially	8	Mature green	26.6	12.8
Wax removed on 9th day, analysed 1 day later	10	Mature green	8.9	22.1

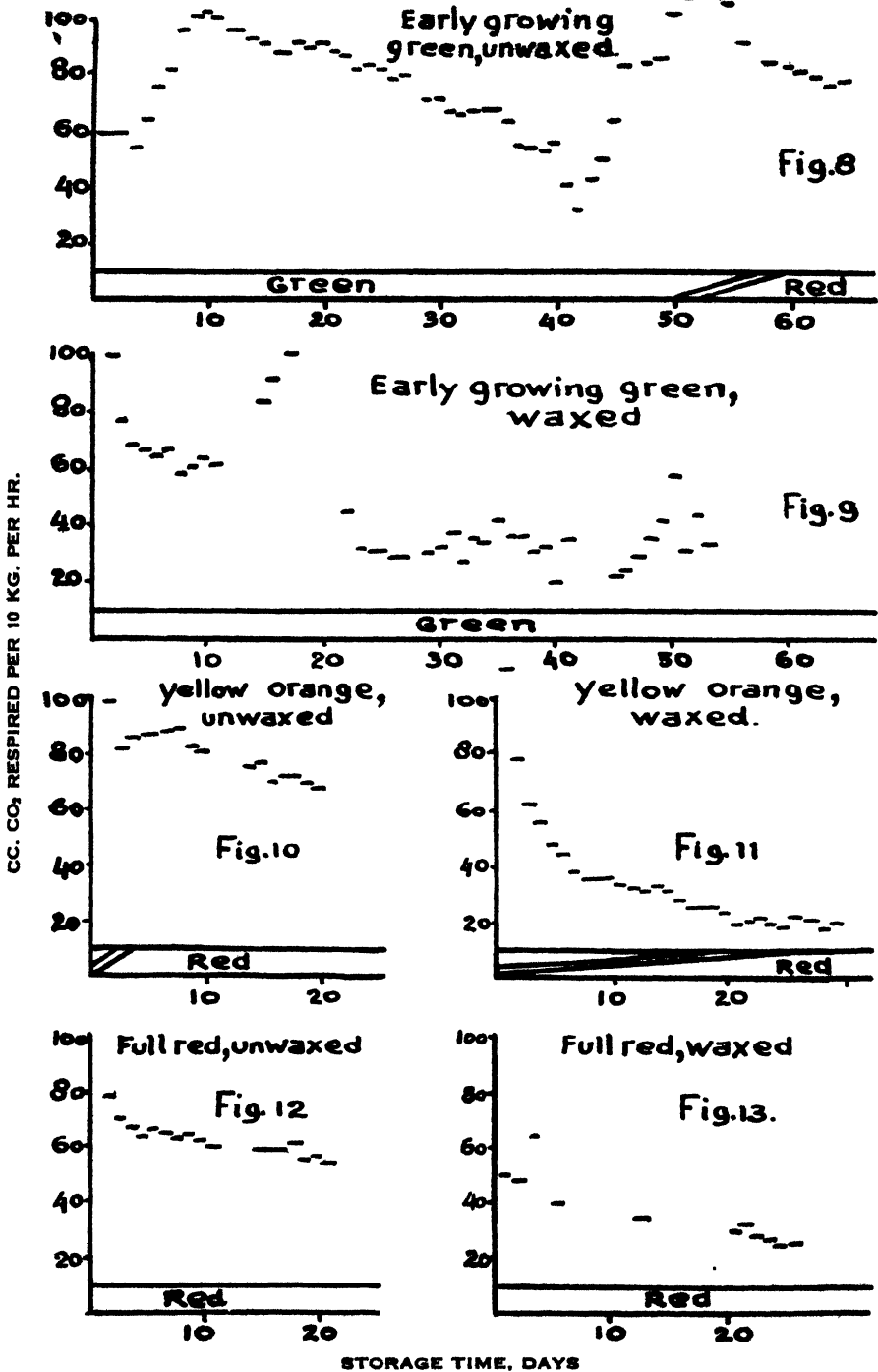
In unwaxed fruits with an initial carbon dioxide content of 7% and oxygen content of 19.3%, the percentage of each increases slightly during storage. During ripening the rising rates of carbon dioxide emission are accompanied by a rising internal carbon dioxide content.

Fruits waxed as soon as picked show a marked increase in carbon dioxide content and a decrease in oxygen content during one week's storage. On removing the wax from the stem scar the carbon dioxide content decreases and the oxygen content increases to such an extent that within 24 hr. from the time of wax removal the internal atmosphere is practically the same as in fruits that had never been subjected to the wax treatment.

This shows beyond doubt that the high "respiration" value observed immediately after wax removal is caused by a liberation of carbon dioxide held in the tissues. Such an early establishment of a new equilibrium indicates a low retentive capacity of the tomato fruit for accumulated carbon dioxide. Measurements of the rate of emission from unwaxed fruits (apparent respiration) thus should give a reasonably accurate indication of the true rate of respiration. Where there have been after-effects of the waxing treatment, i.e., failure to recover their conventional ripening and respiratory behaviour, the previous internal carbon dioxide content rather than the retention of carbon dioxide after wax removal is held to be responsible.

The Effect of Wax Application on Fruits Picked at Various Stages of Maturity

Figs. 9, 5, 11, and 13 are typical of the respiration records obtained on applying a wax seal to the calyx scar of fruits picked and stored at the "growing green" stage (weight, 15 to 25 gm.) and when mature green, yellow orange, and full red, respectively. The progressive changes in the respiration rate of fruits picked at these same maturities and stored without waxing the stem scars are shown in Figs. 8, 1, 10, and 12.



FIGS. 8 TO 13. Effect of waxing on respiration rate of early growing green, yellow orange, and full red tomato fruits in storage.

With fruits picked at the mature green and later stages, the general effect of wax application is the same. Beginning at initial rates that depend on the intensity of respiration on the vine at the time of isolation (1, 7, 14, 16), the rate of carbon dioxide output then is lowered progressively because of the modification of the internal atmosphere. The colour changes associated with ripening are seriously inhibited or they may be stopped entirely. In two or three weeks' time, a steady minimal rate of respiration is attained; this is usually of the same order for fruits picked at all stages of maturity from mature green onwards.

The respiration of fruits picked and stored at various growing green stages has been dealt with rather extensively by Walford (16). He regularly found two distinct peaks in the respiration rate if the fruits ripened in storage. He attributed the first of these to an accumulation of sugars at the expense of reserve carbohydrates. As the second peak in respiration rate was associated with the ripening colour change, Walford decided that it was the usual respiratory climacteric.

The respiration records of waxed growing green fruits, as typified in Fig. 9, show differences that distinguish them sharply from those of unwaxed sister fruits (Fig. 8). Waxed growing green fruits have shown consistently higher initial rates of respiration than have the unwaxed individuals, a phenomenon that has never occurred with fruits picked at later stages. In some instances the first peak in the rate of carbon dioxide output of unwaxed fruits (Fig. 9) appears to be abolished entirely by waxing. More often this respiration phenomenon has been postponed and the amount of extra carbon dioxide evolved in it has been diminished because of its shorter duration in time. However, the peak values that are attained in this phase of the records of waxed growing green fruits are frequently quite as high as those shown in the first respiration peak of unwaxed sister fruits.

Until more is known of the concurrent metabolism of waxed fruits of this physiological stage, the peak shown in Fig. 9 is taken to be the initial peak in respiration rate of Fig. 8 as modified by waxing. In some instances waxing has resulted in its complete suppression; more often it has occasioned a postponement of the sharp rise and an abbreviation of the phase of high carbon dioxide production as a whole. Thereafter the respiration rate falls, finally attaining a level approximately the same as that of sealed fruits picked at later stages of maturity.

Tomato fruits picked when only one-quarter grown (25 gm. or less) have never failed to ripen in storage at 12.5° C. so long as the stem scar area was not sealed with wax. When the stem scar area was waxed the fruits have always become subject to one or more of the physiological disorders described below, before there was any trace of ripening coloration.

It has been found (unpublished data) that during the initial rise in respiration rate (Fig. 8) of unwaxed early growing green fruits there is a marked increase in both sugar and acid content and a decrease in protein nitrogen.

At the same time the seeds are enlarging so that as ripening commences the seeds have become full sized and the gelatinous pulp in which they are embedded becomes deliquescent.

The initial increase in respiration is caused evidently by the increasing acid and sugar content. Seed development proceeds as when the fruit is growing on the plant and its carbon requirements may explain the decrease in respiration rate prior to the onset of ripening. At the time that seed development is terminated these small fruits then undergo a typical ripening colour change accompanied by a senescent rise and fall in carbon dioxide emission.

The respiration records of fruits picked and stored unwaxed at the mature green stage with the stems either attached or detached deserve further comment (Fig. 1). The initial rate of carbon dioxide output at 12.5° C. is between 40 and 60 cc. of carbon dioxide per 10 kg. per hr. The rate remains steady or decreases slightly until the onset of the senescent rise. Rising rates of respiration are observed for three or four days before external yellowing of the fruit is visible. The rate continues to rise another two to four days until the fruit is yellow orange with green persisting to some extent at the shoulder. The ascending arm of the tomato fruit's climacteric is then of five to seven days' duration, a period that is considerably shorter than this phase of the respiration record of apples and pears at a temperature of 12° C. (9, 11). The maximal values attained are 50 to 100% higher than those shown before the onset of the senescent rise, values between 70 and 100 cc. being usual. In comparison, the apple shows maximal rates of the same order while those of the pear are considerably higher (9, 11).

After reaching the peak of its climacteric the respiration of the tomato now undergoes little change or declines slowly until the fruit is full red; this requires an additional five to seven days beyond attainment of the peak. Softening of the fruit occurs to a variable extent in its subsequent storage and the accompanying respiratory drift is usually a slow decline (Fig. 1) but occasional complications have occurred in the form of plateaus and secondary peaks. Similar complications have been recorded for the McIntosh apple in storage at 22° C. (12).

Owing to high susceptibility to the common storage moulds in this late senescent phase, the storage life of the unwaxed tomato fruit may be terminated at any point along this phase in which the respiratory rate slowly declines. Fruits that have not been attacked by fungi for as long as 50 days after attainment of the peak of the climacteric have shown values of between 40 and 50 cc. of carbon dioxide with succeeding values the same or slightly lower until fungal invasion did occur.

The final rate of carbon dioxide output observed in senescent tomatoes at the time of fungal attack is frequently of the same order as the values shown immediately before the onset of the climacteric. Krotkov (12) has drawn attention to this equivalence of rate attained in the McIntosh apple. Krotkov suggests that these minimal rates observed before and after the senescent rise indicate a critical metabolic state, the first leading to the deep seated

changes associated with the senescent rise and the second leading to the "pathological" death of the fruit.

The changes in sugar and acid content associated with the ripening of unwaxed tomatoes during storage result in similar concentrations of these constituents at the mature green and full red stages (unpublished data). It is possible that this similarity before and after ripening is responsible in part at least for the observed similarity in respiration rate. It is the author's opinion, however, that one should look elsewhere for the metabolic basis of the senescent rise and increased susceptibility to fungi that terminate the mature green and full red stages, respectively.

Sound mature green tomatoes stored at 12.5° C., and 80 to 85% relative humidity, with stems attached, only become susceptible to storage fungi after the fully ripened condition is reached. This increase in susceptibility to storage rots is believed to be associated with the accompanying pectic changes, the hydrolysis of protopectin to soluble pectin allowing the fungal hyphae ready access to the fruit through intercellular invasion.

Sound mature green tomatoes stored under similar conditions but with stem scars waxed do not show the simple relation between degree of ripening coloration and susceptibility to fungi that exists in unwaxed fruits. It has been observed that waxed fruits become soft before ripening with sufficient frequency to account for most of this lack of correlation. If the basis of increasing susceptibility of fully ripened fruits with stem scars unwaxed is attributable to changes in their cell wall constituents, the same physiological basis of susceptibility to fungi is to be expected in effectively waxed fruits. In the first instance, the cell wall changes accompany the ripening colour change. In waxed fruits the ripening colour change is inhibited while the cell wall changes are not, at least in those instances in which fungi invade waxed fruits not of full ripe colour.

Recapitulating, Walford recorded two general types of respiration for fruits picked and stored when either mature green, yellowing, yellow orange, or full red. The conventional types represented the completion of those phases of the climacteric that had not been completed while the fruit was attached to the plant whereas in the anomalous types the initial rates varied with the maturity at picking and subsequently declined to a rate of 20 to 30 cc. per 10 kg. per hr. with a marked inhibition of further colour change.

In all fruits of these same stages of maturity, stored with the stems attached or with the stems removed but the stem scar unwaxed, records of the conventional type have been found. Waxing the stem scars of such fruits with paraffin has resulted in the consistent appearance of the anomalous or auto-gas stored types. In all growing green fruits stored with stem scars unwaxed the type previously reported by Walford (16) was obtained. Waxing the stem scars of such fruits resulted in certain modifications of the "initial peak" in carbon dioxide output and physiological breakdown occurred before the appearance of any ripening colour.

In following Walford's technique of applying wax to the stem scar of all fruits studied, Singh and Mathur (15) record examples of the "auto-gas stored" or anomalous type among fruits picked green. All fruits picked at later stages of ripeness showed distinct peaks in carbon dioxide output that appear to be quite unrelated to any accompanying colour changes. If the internal ripeness of their fruits is indicated accurately by skin colour under their conditions, these records must be interpreted as postclimacteric phases of unsealed or partially sealed fruits. A repetition of their observations on such fruits, sealed and unsealed, should prove of interest.

It has been shown above that the anomalous type owes its characteristics to the presence of the wax seal since removal of the wax causes the immediate disappearance of anomalous characteristics while reapplication of wax causes them to appear once more. The initial values of the rate of carbon dioxide emission immediately after wax application and wax removal and the demonstrated changes in the internal atmosphere that result from wax application and wax removal demonstrates that auto-gas storage is the physiological basis of the anomalous and intermediate storage types.

The Functional Disorders of the Waxed Tomato in Storage

The unusual durability in storage of certain tomatoes of the anomalous or sealed type has made this investigation of considerable interest from a commercial standpoint. Assuredly any cultural or storage practices that appear to have an effect on the mean storage life of fruits are worthy of careful study.

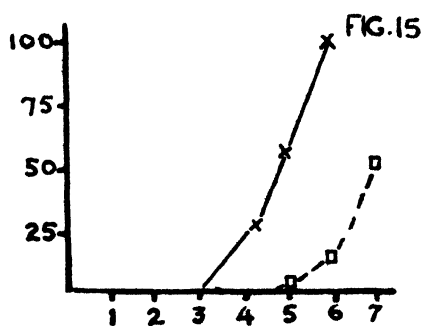
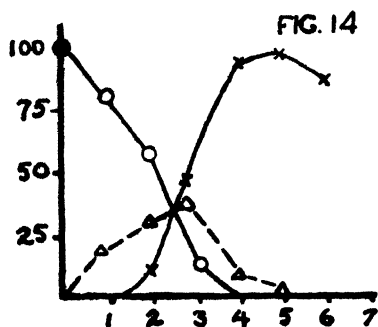
Waxing the stem scar has been recommended elsewhere as a routine commercial practice (4). The experience of the author is that this recommendation is as yet unwarranted. Extension of the storage life of tomatoes by this means appears promising however since the rates of transpiration, respiration, and ripening are greatly diminished. However, at no time has this technique resulted in an increase in the mean storage life in extensive bulk storage trials; this is attributed to undesirable accompaniments which appeared principally in the form of physiological disorders.

The application of wax results first of all in wide differences in ripening rate. This is attributed to a magnification of unavoidable differences in the maturity of the fruits at picking and in the degree of sealing effected by waxing the stem scars. Waxed tomatoes also show great differences in the rate at which different parts of the same fruit attain ripe colour. It follows that some method of overcoming this induced heterogeneity (5) should be found before attempting the waxing technique on a commercial scale.

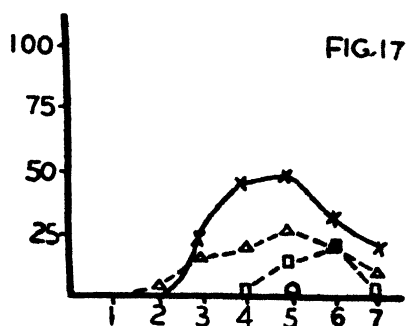
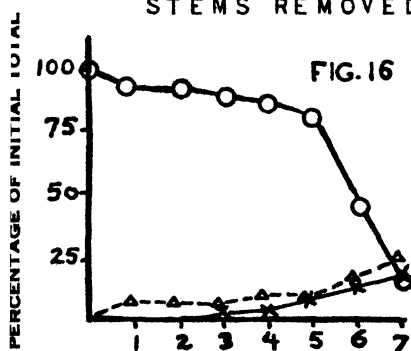
Several physiological disorders, previously unrecorded, have been observed as detrimental accompaniments of the waxing technique. A description of these disorders is included below. The numbers of fruits that may be expected to become affected with them also are indicated for different waxing treatments (Figs. 14 to 19).

Premature softening is characterized, as its name implies, by a serious loss of firmness before the initiation or during early stages of the ripening colour

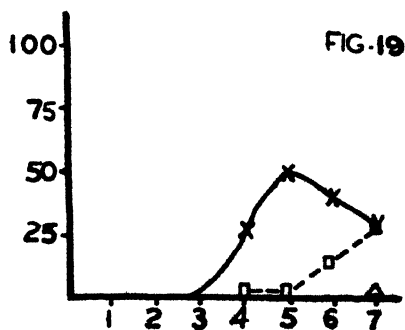
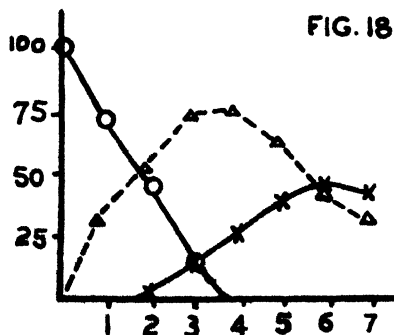
STEMS ATTACHED, UNWAXED



STEMS REMOVED, WAXED AT MATURE GREEN



STEMS REMOVED, WAXED AT YELLOW ORANGE



STORAGE TIME, WK.

FIGS. 14, 16, AND 18. Effect of waxing on ripening behaviour of tomato fruits at 12.5° C. Stage of maturity: ○ = mature green; △ = ripening; × = ripe.

FIGS. 15, 17, AND 19. Effect of waxing on the development of disorders. Type of disorder: ○ = surface browning; △ = surface pitting; × = premature or senescent softening; □ = fungal infection.

change. These soft fruits may acquire a dark, sodden appearance over the locules and at the blossom end. On removal to higher temperatures or on removal of the wax seal, they fail to ripen properly. This disorder is associated with a marked susceptibility to the common storage moulds.

Surface pitting first becomes evident as small white pockets in the skin of the preclimacteric fruit, usually near the blossom end. At later stages more and more of the fruit surface shows this disorder with the small sunken areas tending to merge. Ripening has not been observed in fruits showing these symptoms on removal of the wax or on storing at 70° C. The use of oiled wraps in a number of bulk storage trials has had no effect whatsoever on the number of fruits that become pitted. Attempts to isolate causal organisms from the pits have failed but pitted fruits are noticeably susceptible to fungal attack particularly in the sunken areas. This disorder evidently involves a preliminary physiological breakdown of the skin cells followed by their desiccation and collapse.

Surface pocketing involves lesions of larger area than does pitting. The lesions are deeper and are not necessarily associated with a whitish appearance. In the particular storage experiment recorded below this disorder is included under "surface pitting".

Surface browning appears as superficial brown blotches or smears at or near the blossom end, gradually spreading upwards. This disorder is not related to a collapse of the skin tissue nor is it associated with any noticeable increase in susceptibility to fungus. On removal of the wax seal the result has been a completion of the ripening colour change in all parts of the fruit except at regions showing the injury. Here the brown colour of the skin persists while the underlying wall tissue ripens to a variable extent. The respiratory drift of such a fruit has been followed before and after wax removal, and a month after wax removal when the stem scar has been once more sealed with wax. The respiration record obtained during this manipulation of wax has corresponded in every detail with that shown in Fig. 7 for normal fruits, the rise in respiration rate after wax removal being associated with the ripening colour change.

Fruits picked at any stage of maturity and stored at 12.5° C. with stem scars unwaxed are not subject to the above disorders. Fruits stored at the mature green stage, with the stem scars waxed, may show any of them. However, if fruits are allowed to remain on the plant until yellow orange or full red and then are stored with stem scars waxed, pitting and browning do not occur to any extent.

The above observations suggested that a feasible method of controlling pitting, browning, and unequal ripening rates might be to postpone wax application until the fruits attain considerable ripening coloration in storage. Accordingly an experiment was conducted using different varieties of tomatoes to ascertain if such a method of control was feasible.

In this experiment the fruits were divided into three uniform lots. The first lot was stored with the stems attached throughout, thus serving as a

check. The stems were removed from the second lot and before being stored the scars were covered carefully with paraffin. The third lot was stored with the stems attached until the fruits coloured to yellow orange, thereupon their stems were removed and the stem scars were waxed carefully.

Figs. 14 to 19 give the results obtained with the Grand Rapids variety, using fruits of a 1940 early spring greenhouse population. The general picture for this variety as regards ripening rate and incidence of fungal and physiological disorders applies equally to the other varieties and hybrids tested simultaneously. In the experiment under discussion fruits were not discarded until attacked by fungi. Thus, owing to the removal of such fruits, Fig. 17 shows a decline in the numbers of fruits affected with presenescent softening, pitting, and browning. The actual wastage is not quite as great as the data indicates since all values for wastage represent "total" counts for each disorder and some fruits showed more than a single form of breakdown.

Figs. 14, 16, and 18 were obtained by counting the numbers of fruits of each ripening class after the storage periods indicated and by expressing these counts as percentage of the initial total. After four weeks' storage, practically all unwaxed fruits (Fig. 14) were either ripening or ripe while 85% of the fruits waxed initially were still mature green (Fig. 16). At five weeks all unwaxed fruits were full red while the "waxed initially" lot showed only 10% either orange or red.

Figs. 16 and 17 show the extreme heterogeneity to be expected after five to seven weeks' storage within a population of fruits picked mature green and stored at 12.5° C. with stem scars sealed carefully with paraffin. Fruits of all degrees of ripeness from mature green to full red are seen to be present in large numbers and, in each ripening class, storage disorders are serious.

The application of wax to the stem scar when the yellow orange stage is attained in storage results of course in a marked increase in the number of fruits at this stage of ripening. The wax seal serves to maintain the fruit at the stage at which the wax is applied by inhibiting further ripening coloration. Mention was made earlier of the heterogeneity of fruits waxed at the mature green stage. This does not apply to the same extent to fruits allowed to ripen partially before wax is applied but the greatest homogeneity in this respect is still found in lots stored with the stems attached throughout. The first evidence of wastage among fruits of the check lot was senescent softening of the full red fruits with negligible amounts of wastage of other kinds up to six weeks' storage.

The original purpose of this bulk storage experiment was to test the efficacy of delayed waxing in controlling pitting and browning and heterogeneity within the population with respect to ripening rate. Fig. 19 shows that delayed waxing will eliminate these undesirable accompaniments of earlier wax application. But unfortunately, delayed waxing served to augment the numbers of fruits becoming soft prematurely and the severity of this effect discounts the advantages cited above for such a practice.

The waxing of the stem scar has not resulted in any control whatsoever of fungal attacks at either the stem scar or other areas. The greatest freedom from fungal attack is found consistently in unwaxed fruits stored with the stems attached. Application of a wax cover has even been observed to favour the growth of moulds in many instances. As suggested by Claypool (6) the effect is evidently caused by the higher relative humidity existing immediately under the wax.

In view of the undesirable effects of waxing cited above, it is considered inadvisable at present to recommend wax application to the stem scars as a commercial practice. A treatment of the stem scar area that results in an ideal restriction of carbon dioxide emission and in a complete restriction of water loss will be sought in further experiments. In the meantime Canadian grown tomatoes of high quality may be held satisfactorily for five weeks by storing at the mature green stage with stems attached, at a temperature of 10 to 12.5° C., and at a relative humidity of 80 to 85%.

Acknowledgments

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